JOURNAL OF DAIRY SCIENCE

VOLUME XXIII MAY, 1940 NUMBER 5

# THE CAROTENE CONTENT OF SEVERAL HERBAGES DURING THE GROWING SEASON*

W. W. SNYDER AND L. A. MOORE

*Section of Dairy Husbandry, Michigan Agricultural Experiment Station, East Lansing*

During the past few years an increasing tonnage of various forage crops is being preserved by the ensiling process. Very few studies, however, have been made to determine at what stage of growth these crops contain the greatest amount of carotene. If this information were available it might be possible to ensile certain crops when the carotene content is high. A high carotene roughage is especially desirable in feeding dairy cattle in order to produce a milk of high vitamin A content.

Virtanen and co-workers (10) reported that the total quantity of carotene of a plant increased rapidly up to the time of blooming and then diminished as the plant matured. In a later report Virtanen (9) indicated that carotene attained a maximum value just before or at the beginning of flowering, but that it may be increased by proper and adequate fertilization or it may be decreased by the same factors that retard growth. Hauge (4) found that young alfalfa, 10 to 12 inches high, contained almost twice as much vitamin A as did the alfalfa in full bloom. Hilton, Hauge and Wilbur (5) stated that young alfalfa, 10 to 12 inches high, contained 90 units of vitamin A per gram but in the bloom stage it contained only 70 units. They also noted that soybeans, 12 to 15 inches high, contained 54 units of vitamin A while the more mature plant, as cut for hay, contained only 30 units.

By studying the carotene content of some South African feeds Myburgh (6) found that the carotene content of pasture plants diminished rapidly as the plants matured or as they became dry during winter or during periods of drought. His results on pasture grass during the four seasons for two years showed that the grass was relatively high in carotene in summer and autumn but rather low during the early spring and late winter.

In Arizona blue grama range grass lies in the dormant stage during the winter. In the summer a new growth is begun which is stimulated by rains. By the middle of October the grass begins to dry up again. Smith and Stanley (8) determined the vitamin A value of this grass at different stages of growth. They noted that a sample collected August 2 was a very potent

Received for publication October 17, 1939.
* Journal article No. 398 n.s.

source of vitamin A, by September 20 it was only one-half as rich, and when sampled in November, 100 times as much of the grass was required to produce the same rate of gain in rats as that cut in August.

Atkeson and colleagues (1) determined the carotene content of each of 13 pasture plants three or four times during the growing season, and observed that the plants were relatively high in carotene during the early summer, although there was considerable variation. Carotene decreased during the hot summer months but after the fall rains most plants reestablished their carotene content similar to early summer values. Big blue stem and buffalo grass were notable exceptions in that they showed a decrease as the season progressed.

Esselen and co-workers (2) found that the vitamin A content of the maize plant increased as the plant reached full growth after which there was a marked loss of vitamin A.

Watkins (11) studied the monthly variation in carotene content of black grama and mesa dropseed range grasses grown in New Mexico. His results indicate that rains which stimulate new growth also cause an increase in the carotene content of the grasses. Both grasses were moderately high in carotene during the growing season. The mesa dropseed lost all of its carotene soon after the fall freezes ended the growing season; however, the black grama grass, whose upright stems remained partly green throughout the winter contained an amount of carotene that seemed to be ample to satisfy the vitamin A requirements of range cattle.

## EXPERIMENTAL

The purpose of this investigation was to study the weekly variation in the carotene content of various herbages throughout their growing season. The herbages studied included the following: a mixture of the Grimm and Harrison varieties of alfalfa, Canadian brome grass, corn leaves from Michigan hybrid corn 561, Iagold variety of oats, Manchu variety of soybeans, biennial white sweet clover, and Sudan grass. These herbages were grown on soil of average fertility on the college farm, except the sweet clover which was growing voluntarily on a vacant lot near the college. The alfalfa and brome grass were taken from a field with a good stand of an alfalfa-brome mixture. Samples for carotene analysis were collected once a week from early growth until the crop was harvested or cut for hay. Plants of average size were selected at random and their height ascertained. The appearance of blossoms or development of seeds was also noted at the time of sampling, so that the stage of maturity could be determined. The plants were cut off about an inch above the ground, wrapped in heavy paper and immediately taken to the laboratory.

In the laboratory, a representative portion of the plants was ground through a food chopper and thoroughly mixed in order to obtain a represen-

tative sample. A two to five gram sample was quickly weighed out and immediately covered with alcoholic KOH in a mortar. The sample was placed in the alcoholic KOH as soon as possible to prevent enzymatic or oxidative destruction of carotene. A small amount of fine clear quartz sand was added and the tissue thoroughly ground. The ground sample was then transferred to a 250 ml. Erlenmeyer flask, using sufficient alcoholic KOH to make the transfer complete.

The method used for extracting carotene from the plant tissue was the Guilbert method (3) as modified by Peterson, Hughes and Freeman (7). The carotene concentration of the extract was determined by means of a photo-electric colorimeter. The moisture content of all samples was also determined in order to calculate the results in terms of micrograms of carotene per gram of dry matter. For the moisture determination, five grams of the ground tissue were weighed out and dried for three hours in a drying oven at 100° C.

## RESULTS

Data are presented in table 1 showing the weekly variation in the carotene content of the herbages studied during their growing season of 1938. The data include the results for three crops of alfalfa, two crops of brome grass, corn leaves during the latter part of growth, one crop of oats, one crop of soybeans, two crops of Sudan grass, and one crop of sweet clover.

The first sample of alfalfa was collected April 25 at which time it contained 432 micrograms of carotene per gram of dry matter and when it was cut for hay June 20 this value was only 171 micrograms. The decrease in carotene was not gradual and progressive for the first crop. Sampling of the second growth was begun 17 days after the first crop was cut for hay at which time the carotene content was 318 micrograms. This value dropped progressively to 132 micrograms by August 16 when the second growth was cut for hay. The carotene content of the first sample of the third crop was 367 micrograms, and five weeks later when in the three-fourths bloom stage this value was still 284 micrograms. The third crop showed a gradual decrease in carotene as the plants matured except for one sample taken September 21 at which time the value was slightly higher than that for the previous week.

Sampling of the brome grass was begun April 25 at which time it contained 453 micrograms of carotene per gram of dry matter. When the grass was cut for hay June 6, the carotene content was only 141 micrograms. Fifteen days later the second growth reached a height of seven inches and the carotene content was 466 micrograms. The carotene increased to 518 micrograms by the following week, but thereafter decreased. When the second growth was cut for hay the carotene content was still 317 micrograms. This value was more than twice the value of the first crop when cut for hay.

The first sample of corn leaves was collected as the tassels appeared on the corn, and at that time the carotene content was 482 micrograms per gram

TABLE 1

*Carotene content of several herbages in micrograms per gram of dry matter, during their growing season*

| Date | Height | Dry matter | Carotene | Remarks |
|---|---|---|---|---|
| | *inches* | *per cent* | *micrograms* | |
| **Alfalfa—first crop** | | | | |
| 4–25–38 | 5 | 20.8 | 432.0 | |
| 5– 3–38 | 8 | 20.4 | 397.0 | |
| 5– 9–38 | 11 | 22.9 | 269.0 | |
| 5–16–38 | 13 | 24.2 | 278.0 | |
| 5–23–38 | 18 | 19.0 | 295.0 | raining |
| 5–29–38 | 23 | 20.0 | 233.0 | " |
| 6– 6–38 | 24 | 22.0 | 188.0 | few blossoms |
| 6–13–38 | 28 | 25.7 | 150.0 | early bloom |
| 6–20–38 | 31 | 27.1 | 171.0 | half bloom |
| 6–20–38 | | | | cut for hay |
| **Alfalfa—second crop** | | | | |
| 7– 7–38 | 11 | 19.4 | 224.6 | |
| 7–11–38 | 13 | 19.1 | 301.9 | |
| 7–18–38 | 17 | 26.7 | 272.7 | few blossoms |
| 7–26–38 | 20 | 32.3 | 221.5 | early bloom |
| 8– 2–38 | 21 | 32.4 | 212.8 | half bloom |
| 8– 9–38 | 22 | 32.9 | 169.9 | ¾ bloom |
| 8–16–38 | 22 | 31.8 | 116.0 | ¾ bloom |
| 8–17–38 | | | | cut for hay |
| **Alfalfa—third crop** | | | | |
| 8–23–38 | 15 | 21.1 | 366.7 | |
| 8–29–38 | 18 | 21.6 | 368.2 | |
| 9– 5–38 | 20 | 24.6 | 340.6 | few blossoms |
| 9–13–38 | 21 | 28.8 | 311.7 | early bloom |
| 9–21–38 | 22 | 27.5 | 321.1 | half bloom |
| 9–26–38 | 22 | 29.9 | 283.6 | ¾ bloom |
| **Brome grass—first crop** | | | | |
| 4–25–38 | 3 | 23.8 | 453.0 | |
| 5– 2–38 | 5 | 22.8 | 377.0 | |
| 5– 9–38 | 11 | 23.6 | 284.0 | |
| 5–16–38 | 15 | 22.5 | 311.0 | |
| 5–23–38 | 21 | 19.8 | 293.0 | raining |
| 5–29–38 | 26 | 17.1 | 306.0 | " |
| 6– 6–38 | 34 | 22.0 | 157.0 | few heads out |
| 6–13–38 | 36 | 30.2 | 144.8 | heads out |
| 6–20–38 | 45 | 32.0 | 140.6 | cut for hay |
| **Brome grass—second crop** | | | | |
| 7– 5–38 | 7 | 15.1 | 466.0 | |
| 7–11–38 | 10 | 13.8 | 517.6 | |
| 7–18–38 | 15 | 18.0 | 418.5 | |
| 7–25–38 | 19 | 24.7 | 412.9 | |
| 8– 2–38 | 20 | 25.5 | 390.8 | |
| 8– 9–38 | 23 | 29.6 | 305.2 | few heads |
| 8–16–38 | 23 | 24.4 | 317.1 | raining |
| 8–17–38 | | | | cut for hay |

TABLE 1.—(*Continued*)

| Date | Height | Dry matter | Carotene | Remarks |
|---|---|---|---|---|
| | *inches* | *per cent* | *micrograms* | |
| **Corn leaves** | | | | |
| 8– 9–38 | 105 | 25.4 | 481.6 | tassels out |
| 8–17–38 | 110 | 22.9 | 615.7 | pollen ripe |
| 8–24–38 | 112 | 27.5 | 646.1 | early milk |
| 8–30–38 | 115 | 25.6 | 561.2 | early milk |
| 9– 5–38 | 118 | 29.8 | 539.1 | late milk |
| 9–13–38 | 118 | 24.2 | 516.5 | dough stage |
| 9–21–38 | 118 | 27.7 | 361.0 | glaze stage |
| 9–26–38 | 116 | 31.3 | 243.3 | grain hard |
| 10– 3–38 | 118 | 59.4 | 68.4 | corn ripe |
| 10–11–38 | 118 | 90.0 | 25.4 | leaves dry |
| **Oat plant** | | | | |
| 5–11–38 | 6 | 14.3 | 525.0 | |
| 5–18–38 | 8 | 14.9 | 442.9 | |
| 5–25–38 | 10 | 14.7 | 449.0 | |
| 6– 1–38 | 15 | 14.0 | 375.0 | |
| 6– 8–38 | 18 | 16.4 | 219.5 | |
| 6–15–38 | 20 | 17.5 | 260.0 | |
| 6–22–38 | 30 | 19.8 | 140.5 | head in boot |
| 6–29–38 | 34 | 24.5 | 100.4 | head out of boot |
| 7– 7–38 | 39 | 29.4 | 66.1 | milk stage |
| 7–11–38 | 39 | 30.7 | 54.3 | dough stage |
| 7–18–38 | 39 | 40.0 | 14.7 | late dough |
| 7–25–38 | 39 | 48.7 | 12.6 | ripe |
| 7–27–38 | | | | harvested |
| **Soybean plant** | | | | |
| 6–29–38 | 9 | 22.5 | 279.0 | |
| 7– 5–38 | 14 | 20.3 | 288.8 | few blossoms |
| 7–12–38 | 17 | 21.3 | 299.5 | early bloom |
| 7–19–38 | 21 | 22.5 | 357.8 | few pods |
| 7–26–38 | 22 | 22.6 | 432.2 | more pods |
| 8– 2–38 | 27 | 26.4 | 406.6 | few beans |
| 8– 9–38 | 30 | 25.3 | 334.7 | small beans |
| 8–16–38 | 34 | 24.5 | 248.7 | beans developing |
| 8–23–38 | 36 | 29.0 | 196.9 | " " |
| 8–29–38 | 36 | 29.5 | 193.6 | " " |
| 9– 5–38 | 36 | 30.3 | 163.4 | beans developed |
| 9–13–38 | 36 | 34.5 | 30.5 | yellow leaves |
| 9–21–38 | 37 | 34.5 | 35.4 | beans mature |
| 9–26–38 | 36 | 38.4 | 21.0 | leaves dropping |
| 10– 3–38 | 36 | 66.8 | 7.9 | stalks dry |
| **Sudan grass—first crop** | | | | |
| 7– 7–38 | 18 | 15.6 | 454.7 | |
| 7–12–38 | 34 | 17.1 | 316.9 | |
| 7–19–38 | 43 | 20.4 | 218.5 | |
| 7–26–38 | 48 | 25.0 | 198.4 | heading |
| 7–29–38 | | | | grass cut |
| **Sudan grass—second crop** | | | | |
| 8– 9–38 | 10 | 20.1 | 362.1 | |
| 8–17–38 | 20 | 15.8 | 403.8 | raining |
| 8–24–38 | 36 | 17.9 | 317.9 | heads in boot |
| 8–30–38 | 43 | 18.4 | 229.5 | heads out of boot |
| 9– 5–38 | 46 | 24.0 | 179.3 | seeds developed |
| 9–21–38 | 48 | 25.3 | 112.5 | leaves yellow |
| 9–26–38 | 50 | 36.1 | 57.6 | grass cut |

TABLE 1.—(*Continued*)

| Date | Height | Dry matter | Carotene | Remarks |
|---|---|---|---|---|
| | *inches* | *per cent* | *micrograms* | |
| Sweet clover | | | | |
| 4–27–38 | 7 | 14.6 | 218.0 | |
| 5– 4–38 | 12 | 12.8 | 242.0 | |
| 5–11–38 | 18 | 16.4 | 244.0 | |
| 5–18–38 | 20 | 16.0 | 175.0 | |
| 5–25–38 | 24 | 16.0 | 216.0 | |
| 6– 1–38 | 30 | 17.4 | 208.0 | |
| 6– 8–38 | 37 | 19.2 | 177.0 | few blossoms |
| 6–15–38 | 42 | 20.0 | 172.0 | early bloom |
| 6–22–38 | 66 | 22.8 | 125.0 | full bloom |
| 6–23–38 | | | | cut |

of dry matter. This value increased during the next two weeks to 646 micrograms, after which time there was a progressive decrease in carotene as the plants matured. When the corn was mature enough for husking the carotene content of the dried leaves was only 25 micrograms.

When the oats were six inches high sampling was begun. At this time the carotene content was 525 micrograms per gram of dry matter, and when harvested this value had decreased to 13 micrograms. The results showed a definite downward trend in carotene content as the plants matured, although the decrease was not uniform from week to week. The most notable drop in carotene was during the early growing season until the heads appeared, during which time the original 525 microgram value was lowered to 100 micrograms.

The first values for soybeans were obtained when the plants were nine inches high, at which time the carotene content was 279 micrograms per gram of dry matter. This value gradually increased to 432 micrograms when the plants were 22 inches high and the bean pods were developing. From this stage of maturity on, there was a gradual and progressive decrease in carotene as the plants matured, so that by the time the leaves were yellow the carotene content of the plant was only 35 micrograms.

The carotene analyses for sweet clover are also presented in table 1. The carotene content of the young plants increased progressively to a rather low maximum of 244 micrograms per gram of dry matter when the plants were 18 inches high. After that there was a decrease as the plants matured and when in full bloom the carotene content was only 126 micrograms.

## DISCUSSION OF RESULTS

The results for the herbages studied clearly show that the carotene content per gram of dry matter is much higher when the plants are in the earlier stages of growth than after they reach the stage of maturity at which they are usually harvested. The early growth alfalfa contained more than 300 micrograms of carotene per gram of dry matter, but when cut for hay, the

first crop contained only 171 micrograms and the second crop 133 micrograms. The carotene content of the third crop was considerably higher. However, the samples were taken from a different field so the results may not be directly comparable. Soybeans, on the other hand, when 10 to 15 inches high, contained slightly less than 300 micrograms of carotene per gram of dry matter but this value increased to 432 micrograms when the plants were 22 inches high and by the time the beans were developing, at which stage they are usually cut for hay, this value was approximately 200 micrograms.

First crop brome grass contained 141 micrograms of carotene per gram of dry matter when cut for hay, while the second crop contained 317 micrograms. This difference was probably due to the fact that the first crop developed to a more mature stage than did the second. The carotene content of Sudan grass, 18 to 20 inches high, was slightly more than 400 micrograms but after heading this value dropped to about 200 micrograms. Both crops followed the same rate of decrease. The oat plant when 6 to 10 inches high contained more than 400 micrograms but after heading this value dropped to less than 150 micrograms. The sweet clover used in this investigation was lower in carotene than the other plants studied. The maximum of 244 micrograms per gram of dry matter was noted at 18 inches, which value decreased to 125 micrograms when in full bloom. On the other hand, corn leaves reached a maximum of 646 micrograms when the ears were in early milk stage, which value decreased to 517 micrograms when in the dough stage. As the leaves became yellow the carotene decreased very rapidly to a low value of 25 micrograms.

The decrease in carotene content as the plants matured was not progressive in all cases. During the latter part of May, alfalfa and brome grass, especially, did not show an even rate of decrease. As a matter of fact, in some cases a slight increase in the trend was noted. This was probably due to an increase in the rate of growth of the plants caused by the additional rainfall during that time. A report by the U. S. Weather Bureau revealed that the total precipitation for the month was 5.73 inches, most of which fell during the latter half of the month. This additional rainfall apparently increased the rate of growth which is concomitant with an increased carotene content (10, 11).

Some of the variations in carotene content during the growing season cannot be explained on the basis of differences in the rate of growth. Attention should be called to the fact that the carotene analyses were made on different plants in successive weeks. Thus some of the variations from week to week may have been due entirely to sampling, since it is almost impossible to select random samples under normal growing conditions that are exactly comparable. Most of the carotene of herbages is contained in the leaves (4). Thus in the event of selecting a sample for analysis which contained more stems, the carotene content would be lower, while a plant with more leaves would

contain more carotene. This, and also the fact that plants are biological tissue in which changes are continually taking place, might help explain some of the variations from the normal trends.

The differences in trends for the carotene content of the various plants during growth were interesting. In the case of alfalfa, brome grass, oats, and Sudan grass, the carotene content decreased from the earliest samples until maturity. The second growth of brome grass did show an increase during the first week, but thereafter the regular decrease was noted. The sweet clover showed a gradual increase during the first two weeks after which the trend was downward. In contrast to these plants the soybeans showed a progressive increase in carotene up to and including three weeks' growth after the first blossoms appeared, but thereafter a definite downward trend was noted. In the case of corn leaves, which were used in this investigation, rather than the entire plant, sampling was not begun until the tassels were out, at which time the carotene content was increasing. The increase continued for two weeks, after which a definite and progressive decrease was noted. These variations in trends may be due entirely to plant species differences. It should be mentioned, however, that the blooming process in the case of soybeans is slow and gradual.

From the results obtained it would seem evident that plants should be cut at an early stage of growth in order to obtain the greatest carotene content. However, for ensilage the crop should be cut at that stage of maturity which is complementary to proper preservation in the silo.

## SUMMARY AND CONCLUSIONS

1. Variations in the carotene content of seven herbages were studied during their growing season. These herbages included alfalfa, brome grass, corn leaves, the oat plant, the soybean plant, Sudan grass, and sweet clover.

2. The carotene content of these herbages is much greater during the earlier stages of growth than after they reach the stage of maturity at which they are usually harvested.

3. The carotene content, when calculated in terms of micrograms per gram of dry matter shows a rather progressive decrease as the plants mature except where affected by factors governing the rate of growth.

4. In making hay or silage, in order to obtain the greatest carotene content the plants should be cut at an early stage of maturity.

## REFERENCES

(1) Atkeson, F. W., Peterson, W. J. and Aldous, H. E. Observations on the carotene content of some typical pasture plants. J. Dairy Sc. 20: 557–562. 1937.

(2) Esselen, W. B., Fellers, C. R. and Isgur, B. Vitamins A, C and D in maize as affected by variety and stage of growth. J. Nutrit. 14: 503–511. 1937.

(3) Guilbert, H. R. Determination of carotene as a means of estimating the vitamin A value of forage. Ind. Eng. Chem., Anal. Ed. 6: 452–454. 1934.

(4) HAUGE, S. M. Vitamin A value of alfalfa cut at different stages of maturity. J. Assoc. Official Agr. Chem. 17: 304–307. 1934.

(5) HILTON, J. H., HAUGE, S. M. AND WILBUR, J. W. The vitamin A activity of butter produced by cows fed alfalfa hay and soybean hay cut at different stages of maturity. J. DAIRY SC. 18: 795–800. 1935.

(6) MYBURGH, S. J. The carotene content of some South African feeds. Onderstepoort J. Vet. Sc. 5: 475–482. 1935.

(7) PETERSON, W. J., HUGHES, J. S. AND FREEMAN, H. F. Determination of carotene in forage. A modification of the Guilbert method. Ind. Eng. Chem., Anal. Ed. 9: 71–72. 1937.

(8) SMITH, M. C. AND STANLEY, E. B. The vitamin A value of blue grama range grass at different stages of growth. J. Agr. Res. 56: 69–72. 1938.

(9) VIRTANEN, A. I. Vitamins in plants. Nature 137: 779–780. 1936.

(10) VIRTANEN, A. I., HANSEN, S. V. UND SAASTAMOINEN, S. Untersuchungen über die Vitaminbildung in Pflanzen. I. Biochem. Ztschr. 267: 179–191. 1933.

(11) WATKINS, W. E. Monthly variation in carotene content of two important range grasses, *Sporobolus flexuosus* and *Butelona eriopoda*. J. Agr. Res. 58: 695–699. 1939.

NOTE: Since this paper was prepared three papers by F. E. Moon, dealing with similar subject matter, have appeared and should be noted. They are: 1, The composition of grass at various stages of maturity, and the changes occurring during haymaking, with particular reference to carotene content. The Empire Journal of Exp. Agr. 7: 225–234, 1939. 2, The carotene content of some grass and clover species, with a note on pasture weeds. The Empire Journal of Exp. Agr. 7: 235–243, 1939. 3, The influence of manurial treatment on the carotene content of poor pasture grass and on the relationship of this constituent to the ash and organic fractions. Journal of Agr. Sc. 29: 524–543. 1939.

# ASCORBIC ACID AND OXIDIZED FLAVOR IN MILK. II. THE EFFECT OF VARIOUS HEAT TREATMENTS OF MILK UPON THE STABILITY OF ASCORBIC ACID AND UPON THE DEVELOPMENT OF THE OXIDIZED FLAVOR[1]

ERLAND C. GJESSING[2] AND G. M. TROUT

*Department of Dairy Husbandry, Michigan Agricultural Experiment Station, E. Lansing, Michigan*

The stability of ascorbic acid in milk seems especially important inasmuch as normal milk is not an abundant source of this vitamin. Being a reducing agent the presence of ascorbic acid influences oxidative changes in milk and thus has a bearing upon milk flavor. Within recent years many data have been presented showing factors which affect the ascorbic acid of milk and which affect the development of the oxidized flavor.

Kende (8) demonstrated that heating milk to 85° C. (185° F.) for five minutes inhibited the development of the oxidized flavor while pasteurization at 63° C. (145.4° F.) for 30 minutes was ineffective. He observed that 0.05–0.10 mg. of $CuSO_4$ per liter was sufficient to cause the oxidized flavor in raw milk or milk pasteurized by the low-temperature holder process, while 24–40 times that quantity had no effect on milk pasteurized at the high temperature. His studies led him to conclude that milk highly susceptible to oxidation would oxidize only in the presence of an external oxidative agent (*e.g.*, metal salts, copper particularly) and these acting only in the presence of an organic ferment, which he named "oleinase." The high-temperature exposure inactivated the "oleinase" thus inhibiting the development of the oxidized flavor.

With some modification as to the time and temperature of heating, Guthrie and Brueckner (6), Greenbank (5), Thurston (12), Chilson (2), Sharp, Trout, and Guthrie (10), Dahle and Palmer (3), and Gould and Sommer (4) noted also the inhibitory effect of high heat treatments of milk on the development of the oxidized flavor. Brown, Thurston, and Dustman (1) showed that the time of copper contamination with respect to the pasteurization exposure was an important factor in the development of the oxidized flavor.

Chilson (2) added reducing agents, ascorbic acid, elone and hydroquinone to milk and noted that their presence in sufficient quantities prevented the development of the oxidized flavor, for seven days, in milk which

Received for publication October 23, 1939.

[1] Authorized as Mich. Agr. Exp. Sta. Jour. Article No. 397 (n.s.)

[2] The data presented in this paper are from a thesis submitted to the Graduate School of the Michigan State College in partial fulfillment of the degree of Master of Science, June, 1938.

ordinarily developed a very bad oxidized flavor within three days. He noted also that the addition of ascorbic acid to milk gave it a pleasing flavor, seemingly better than the original milk before it became oxidized. Fortified milk having 92.6 mg. of ascorbic acid per liter had only 85.2 mg. per liter when pasteurized at 143° F. (61.6° C.) for 30 minutes, whereas raw milk fortified with 150 mg. of ascorbic acid per liter retained all the ascorbic acid upon heating to 200° F. (93.3° C.) for a similar period.

King and Waugh (9), Whitnah, Riddell, and Caulfield (13), Sharp, Trout, and Guthrie (10), and Dahle and Palmer (3) observed less destruction of ascorbic acid when milk was heated out of contact with copper at temperatures of 160° F. (71.1° C.) or above than when holder pasteurized at 143–145° F. (61.6–62.8° C.).

Hand, Guthrie, and Sharp (7) demonstrated that holder pasteurized milk voided of oxygen by vacuum cooling neither decreased the ascorbic acid content nor developed the oxidized flavor after holding seven days although 0.1 mg. copper per liter was added. In fact, pasteurized milk subjected to the vacuum cooling treatment was much higher in ascorbic acid after holding than the raw milk held for the same length of time.

Gould and Sommer (4), studying the effect of heat on milk with special attention to the cooked flavor, found that the cooked flavor of milk was caused by the formation of sulphides which occurred when milk was subjected to sufficiently high temperatures or to other changes occurring simultaneous to this formation. They found that temperatures sufficiently high to cause a cooked flavor also largely inhibited the development of the oxidized flavor. However, when the addition of copper followed the heating process, no relationship between the cooked and the oxidized flavor was observed. They believed that in addition to being responsible for the cooked flavor of milk, the presence of these sulphides might explain the heat retardation effect resulting in the prevention of the development of the oxidized flavor, an effect attributed by Kende (6) to be due to inactivation of the organic ferment which he designated "oleinase."

## EXPERIMENTAL

The milk used in these studies was machine-drawn into an aluminum container from which it was poured into glass bottles and cooled immediately. The samples were processed in glass, using laboratory equipment. The holding exposures employed were 63° C. (145.4° F.) and 75° C. (167° F.) for 30 minutes and 65 (149), 70 (158), 75 (167), 80 (176), 85 (185), and 90° C. (194° F.) for ten minutes. Samples were flash pasteurized also at ranges of temperature from 60–97° C. (140–206.6° F.) for five to fifteen seconds by drawing the milk, using low vacuum, through glass tubing submerged in hot and cold water baths for appropriate heating and cooling. Thermometers placed in the line of flow indicated the desired

temperatures. The milk, stored at 5° C. (41° F.), was titrated daily for ascorbic acid, titrations being made from portions of each sample using 2–6 dichlorophenolindophenol according to the rapid method of Sharp (1).

Copper was added to the milk in varying amounts before or after pasteurization as the experiment dictated.

After sufficient storage the milk was examined organoleptically for flavor.

For experiment 1, individual samples of milk were obtained from 20 cows of the college herd and were cooled and treated at once. Each sample was divided into five lots: one lot served as a control; two lots were pasteurized at 63° C. (145.4° F.) for thirty minutes, after which 0.13 mg. of copper per liter was added to one lot; the two remaining lots were pasteurized at 75° C. (167° F.) for thirty minutes, after which copper was added to one lot as above.

In experiment 2, samples of milk from six individual cows were flash pasteurized at various temperatures from 60–97° C. (140–206.6° F.) and cooled within a period ranging from five to fifteen seconds.

The milk used in experiment 3 was obtained from three cows, numbers 30, 111, and 116, two Jerseys and a Guernsey, respectively. The milk was divided into two groups, I and II, of six lots each, A, B, C, D, E, and F. The lots were again divided into six portions, 1, 2, 3, 4, 5, and 6 of 200 ml. each. To the six 200 ml. portions copper was added at the rate of 0, 0.13, 0.26, 0.39, 0.52, and 0.65 mg. per liter, respectively. The copper was added to the milk comprising Group I before heating and to that of Group II after heating. Lots A, B, C, D, E, and F of both the Groups I and II were heated for exactly ten minutes at 65 (149), 70 (158), 75 (167), 80 (176), 85 (185), and 90° C. (194° F.), respectively. The milk was treated and the ascorbic acid value determined the same day as milking as well as after various storage periods.

## RESULTS

1. *The effect of copper upon the stability of ascorbic acid and upon the development of the oxidized flavor when the milk was pasteurized for 30 minutes at high and at low temperature.*

The catalyzing effect of copper upon the oxidation of ascorbic acid and upon the oxidation of the fatty constituents of milk was studied in individual samples of milk from 20 cows of the college herd.

The data obtained are presented in tables 1 and 2, and are shown graphically in figure 1.

An examination of the data of table 1 shows the catalyzing effect of copper upon the oxidation of ascorbic acid when the milk was pasteurized at 63° C. (145.4° F.) for thirty minutes. This effect was very marked after the second and third days. On the fourth day no appreciable amount of

TABLE 1

*The ascorbic acid content and the development of the oxidized flavor in milk from individual cows when the milk was holder pasteurized, had copper added, and was stored at 5° C. (41° F.), May, 1937*

| Cow No. | Ascorbic acid (mg. per liter) and oxidized flavor* in | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Raw milk | | | | | Holder pasteurized milk (63° C. (145.4° F.)—30 min.) | | | | | | | |
| | | | | | | No copper added | | | | Copper added (0.13 mg. per liter) | | | |
| | 1st day (control) | 2nd day | 3rd day | 4th day | Oxidized flavor on 4th day | 2nd day | 3rd day | 4th day | Oxidized flavor on 4th day | 2nd day | 3rd day | 4th day | Oxidized flavor on 4th day |
| 13 | 16.4 | 12.5 | 6.8 | 2.9 | – | 10.5 | 9.3 | 5.9 | – | 2.0 | 1.9 | 0.0 | – |
| 30 | 23.6 | 22.6 | 19.6 | 16.7 | – | 22.0 | 19.7 | 12.7 | – | 16.0 | 6.8 | 2.5 | ++ |
| 36 | 18.3 | 11.5 | 2.5 | 2.9 | –† | 9.0 | 2.9 | 1.9 | – | 3.5 | 2.5 | 0.0 | + |
| 37 | 18.3 | 10.5 | 3.4 | 2.5 | – | 9.0 | 3.9 | 1.9 | † | 3.5 | 3.4 | 0.0 | –† |
| 41 | 19.3 | 18.5 | 13.7 | 8.8 | – | 17.5 | 14.7 | 10.8 | – | 11.5 | 2.5 | 0.0 | ++ |
| 42 | 21.7 | 18.1 | 6.8 | 2.5 | – | 14.0 | 8.8 | 5.9 | – | 7.5 | 1.9 | 0.0 | +++ |
| 100 | 24.1 | 20.1 | 12.8 | 5.9 | –† | 20.0 | 14.7 | 12.8 | – | 10.0 | 1.9 | 0.0 | – |
| 111 | 24.5 | 23.5 | 23.1 | 17.7 | +† | 18.5 | 18.7 | 18.7 | † | 16.0 | 5.9 | 2.9 | +++ |
| 116 | 25.5 | 24.1 | 24.1 | 14.7 | – | 23.0 | 20.6 | 18.1 | – | 17.5 | 12.1 | 3.4 | + |
| 117 | 21.2 | 19.5 | 12.8 | 7.8 | +† | 19.5 | 16.7 | 8.8 | +† | 12.0 | 2.9 | 0.0 | ++ |
| 134 | 20.2 | 17.0 | 10.8 | 4.9 | – | 14.0 | 10.8 | 6.8 | – | 5.5 | 1.5 | 0.0 | ++ |
| 142 | 18.8 | 16.0 | 8.8 | 2.9 | – | 16.0 | 10.3 | 5.9 | – | 5.5 | 1.9 | 0.0 | ++ |
| 174 | 16.4 | 12.5 | 7.8 | 1.9 | – | 11.0 | 5.4 | 2.4 | – | 2.5 | 1.9 | 0.0 | –† |
| 180 | 20.7 | 16.0 | 10.8 | 3.9 | – | 18.0 | 12.3 | 9.8 | – | 4.5 | 1.9 | 0.0 | +++ |
| 190 | 21.2 | 17.5 | 12.7 | 8.8 | – | 17.5 | 13.3 | 11.3 | – | 8.0 | 0.9 | 0.0 | +++ |
| 197 | 22.2 | 17.5 | 11.8 | 5.9 | – | 18.5 | 13.7 | 10.8 | – | 4.5 | 0.9 | 0.0 | +++ |
| 237 | 19.7 | 13.0 | 2.5 | 1.9 | –† | 10.5 | 2.9 | 2.9 | –† | 3.0 | 0.9 | 0.0 | + |
| 238 | 22.2 | 17.5 | 13.7 | 5.9 | – | 17.0 | 12.9 | 10.8 | – | 7.0 | 1.9 | 0.0 | ++ |
| 239 | 21.7 | ..... | 6.8 | 1.4 | – | 16.0 | 7.8 | 3.9 | – | 5.0 | 0.9 | 0.0 | + |
| 300 | 18.8 | 16.5 | 13.7 | 3.9 | – | 16.5 | 14.7 | 11.8 | – | 9.0 | 0.9 | 0.0 | +++ |
| Avg. | 20.7 | 17.1 | 11.3 | 6.2 | | 15.9 | 11.7 | 8.7 | | 7.7 | 2.8 | 0.4 | |

* Key: – no oxidized flavor.
† doubtful oxidized flavor.
+ slight oxidized flavor.
++ distinct oxidized flavor.
+++ very strong oxidized flavor.

TABLE 2

*The ascorbic acid content and the development of the oxidized flavor in milk from individual cows when the milk was heat treated at 75° C. (167° F.) for 30 minutes, had copper added, and was stored at 5° C. (41° F.), May, 1937*

| Cow No. | Ascorbic acid (mg. per liter) and oxidized flavor* in | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Raw milk | | | | | Milk heated to 75° C. (167° F.) and held 30 minutes | | | | | | | |
| | | | | | | No copper added | | | | Copper added (0.13 mg. per liter) | | | |
| | 1st day (control) | 2nd day | 3rd day | 4th day | Oxidized flavor on 4th day | 2nd day | 3rd day | 4th day | Oxidized flavor on 4th day | 2nd day | 3rd day | 4th day | Oxidized flavor on 4th day |
| 13 | 16.4 | 12.5 | 6.8 | 2.9 | – | 13.5 | 11.3 | 10.3 | – | 12.0 | 11.3 | 8.8 | – |
| 30 | 23.6 | 22.6 | 19.6 | 16.7 | – | 19.0 | 18.2 | 16.2 | – | 19.5 | 16.7 | 14.7 | – |
| 36 | 18.3 | 11.5 | 2.5 | 2.9 | –† | 11.0 | 9.3 | 8.8 | – | 11.0 | 7.3 | 4.9 | – |
| 37 | 18.3 | 10.5 | 3.4 | 2.5 | – | 12.5 | 10.8 | 9.3 | – | 10.5 | 8.8 | 5.4 | – |
| 41 | 19.3 | 18.5 | 13.7 | 8.8 | – | 15.5 | 12.8 | 12.8 | – | 13.5 | 13.3 | 12.8 | – |
| 42 | 21.7 | 18.1 | 6.8 | 2.5 | – | 14.5 | 13.7 | 11.8 | – | | | | |
| 100 | 24.1 | 20.1 | 12.8 | 5.9 | –† | 17.0 | 16.2 | 14.7 | – | 17.0 | 14.7 | 14.3 | – |
| 111 | 24.5 | 23.5 | 23.1 | 17.7 | +† | 18.5 | 18.7 | 16.7 | – | 18.5 | 17.2 | 15.7 | – |
| 116 | 25.5 | 24.1 | 24.1 | 14.7 | – | 18.0 | 17.2 | 14.7 | – | 19.0 | 17.7 | 17.7 | – |
| 117 | 21.2 | 19.5 | 12.8 | 7.8 | +† | 17.0 | 13.7 | 11.8 | – | 17.0 | 14.3 | 14.7 | – |
| 134 | 20.2 | 17.0 | 10.8 | 4.9 | – | 15.0 | 12.8 | 10.3 | – | 13.0 | 11.8 | 6.4 | – |
| 142 | 18.8 | 16.0 | 8.8 | 2.9 | – | 11.0 | 10.3 | 10.3 | – | 14.0 | 10.8 | 7.8 | – |
| 174 | 16.4 | 12.5 | 7.8 | 1.9 | – | 12.5 | 10.8 | 10.3 | – | 11.0 | 8.8 | 6.4 | – |
| 180 | 20.7 | 16.0 | 10.8 | 3.9 | – | 14.5 | 14.3 | 12.2 | – | 14.0 | 12.8 | 9.8 | – |
| 190 | 21.2 | 17.5 | 12.7 | 8.8 | – | 17.0 | 14.3 | 13.7 | – | 14.5 | 11.8 | 7.8 | – |
| 197 | 22.2 | 17.5 | 11.8 | 5.9 | – | 14.5 | 14.3 | 12.8 | – | 16.0 | 14.3 | 14.3 | – |
| 237 | 19.7 | 13.0 | 2.5 | 1.9 | –† | 13.5 | 11.8 | 9.3 | – | 13.0 | 9.8 | 8.8 | – |
| 238 | 22.2 | 17.5 | 13.7 | 5.9 | – | 15.5 | 14.3 | 12.8 | – | 15.5 | 14.7 | 13.3 | – |
| 239 | 21.7 | ..... | 6.8 | 1.4 | – | 16.0 | 14.3 | 12.2 | – | 15.5 | 14.7 | 12.8 | – |
| 300 | 18.8 | 16.5 | 13.7 | 3.9 | – | 12.5 | 12.8 | 10.8 | – | 15.0 | 13.3 | 10.8 | – |
| Avg. | 20.7 | 17.1 | 11.3 | 6.2 | | 14.9 | 13.6 | 12.1 | | 14.7 | 12.9 | 10.9 | |

* Key: – no oxidized flavor. + slight oxidized flavor.
† doubtful oxidized flavor. ++ distinct oxidized flavor.
+++ very strong oxidized flavor.

ascorbic acid remained. Coincident with the marked decrease in ascorbic acid, the strong development of oxidized flavor was usually noted. However, this observation was not consistent in all samples. Some samples, pasteurized at 63° C. (145.4° F.) and treated with copper, exhibited a very pronounced decrease in the ascorbic acid content without any, or possibly a slight, development of the oxidized flavor. This was observed in milk from cows number 13, 36, 37, 100, 116, 174, 237, and 239. Of these, milk from two cows, number 13 and 100, developed no noticeable trace of oxidized flavor even when contaminated with 0.13 mg. of copper per liter after pasteurization.

The stability of ascorbic acid was slightly less on the average in raw milk over a three-day storage period than similar milk pasteurized by the holder method. A slightly greater loss of ascorbic acid was noted in the pasteurized milk than in the raw milk on the first day of storage. However, on the second day the average losses were about equal. The stability of the ascorbic acid as a result of the high heat treatment was greatly increased over that when the low temperature was employed. Nevertheless, there was a continuous slight decrease in the amount of ascorbic acid present during storage as shown in table 2 and figure 1.

The increased stability of ascorbic acid at the 75° C. (167°F.) exposure over that of the 63° C. (145.4° F.) exposure was shown more strikingly in the cases where copper was added to the milk. The presence of copper resulted in a slight decrease in the ascorbic acid of the milk similarly high

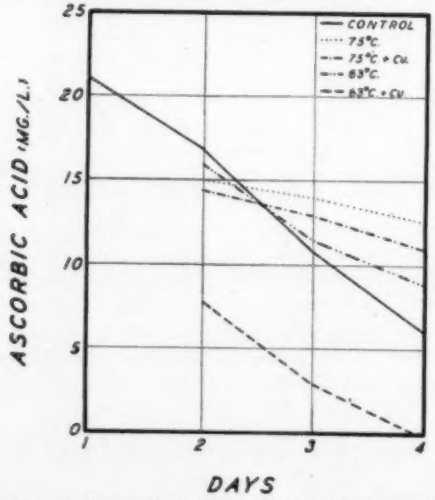



FIG. 1. The relative stability of ascorbic acid in milk pasteurized for 30 minutes at 63° C. (145.4° F.) and at 75° C. (167° F.), respectively, with and without the addition of 0.13 mg. per liter of copper following pasteurization.

heat treated without copper, but was comparatively stable compared to that in milk pasteurized at the lower temperature and held under similar conditions. Oxidized flavor did not develop in the samples thus high heat treated even in the presence of 0.13 mg. of copper per liter.

An examination of tables 1 and 2, shows that milk from certain cows exhibited a natural stability in respect to ascorbic acid, particularly when untreated. This stability was especially noticeable in the case of cows number 30, 111, 116, two Jerseys and a Guernsey, respectively. An examination of the oxidized flavor data showed that the pasteurized copper contaminated milk from these cows was susceptible also to the development of the oxidized flavor. In fact, milk from cow number 111 had already shown, while raw, a tendency to develop the oxidized flavor.

2. *The effect of flash heating of milk at various temperatures upon the stability of the ascorbic acid.*

Individual samples of milk from 10 cows were flash pasteurized at various temperatures from 60–97° C. (140–206.6° F.) and cooled within a period ranging from 5 to 15 seconds. Portions of each sample were titrated daily for seven days for ascorbic acid.

An examination of the data obtained shows that the stability of ascorbic acid in milk from individual cows was not entirely the same under similar conditions of heat treatment; that flash pasteurization temperatures ranging from 85–95° C. (185–203° F.) had the most pronounced stabilizing effect

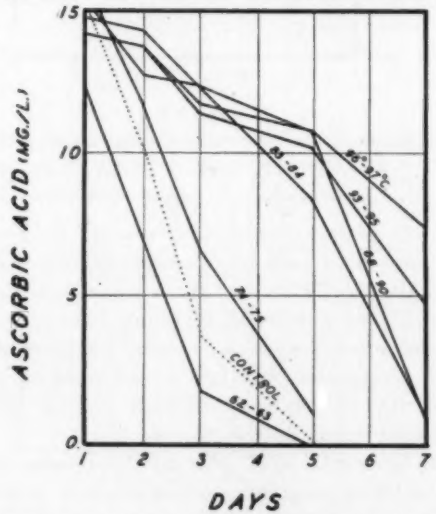



FIG. 2. The effects of various flash pasteurization temperatures (° C.) upon the ascorbic acid content of milk from cow number 144 when stored for several days, June 1937.

upon the ascorbic acid; that flash exposures below 75° C. (167° F.) had slightly less stabilizing effect upon the ascorbic acid than that existing naturally in the raw milk; that the critical flash exposure minima for ascorbic acid stability were between 75 and 85° C. (167 and 185° F.); and that milk having a naturally low ascorbic acid content usually showed greater increased stability of ascorbic acid upon flash heating than did those samples having a naturally high ascorbic acid value.

Data obtained on the milk from two cows which seemed representative of those studied, are shown graphically in figures 2 and 3.

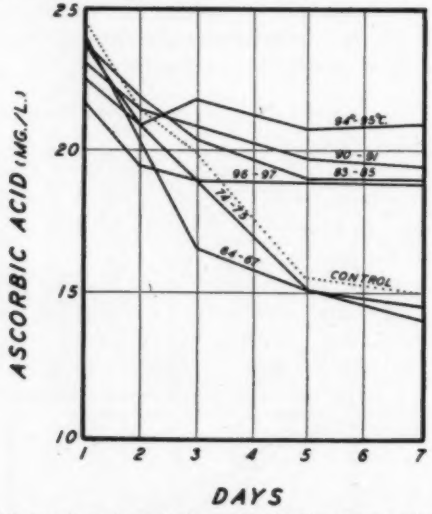



FIG. 3. The effects of various flash pasteurization temperatures (° C.) upon the ascorbic acid content of milk from cow number 116 when stored for several days, June 1937.

3. *The effect of ten-minute exposures at various temperatures upon the ascorbic acid content of milk and upon the development of the oxidized flavor when copper was added before and after heating.*

Mixed milk from cows number 30, 111, and 116 of the college herd was subjected to ten minute exposures at various temperatures. Copper was added to the milk before and after heating. The ascorbic acid values obtained on the milk with the various treatments, initially and after 24 hours storage, are presented graphically in figures 4 and 5.

A temperature of 80° C. (176° F.) for 10 minutes seemed to be an exposure during which marked changes took place in milk as far as factors affecting the stability of ascorbic acid were concerned. When the copper was added to the milk *before* heating, the optimum temperature for the stability of ascorbic acid, regardless of the different amounts of copper

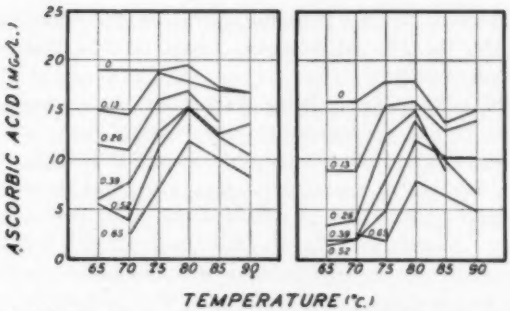



FIG. 4. The stability of ascorbic acid in milk when heated to various temperatures for ten minutes. The milk was contaminated with various amounts of copper (mg. per liter) *before* heating. Values at initial titration and after 24 hours storage respectively.

added, was found to be 80° C. (176° F.). With the addition of increasing amounts of copper, the ascorbic acid content became less regardless of exposure, but 80° C. (176° F.) remained, nevertheless, the optimum temperature of ascorbic acid stability for each individual series. At exposures above 80° C. (176° F.) the ascorbic acid was less stable. However, greater stability was exhibited in the milk heated above 80° C. (176° F.) for ten minutes than when heated to temperatures below 80° C. (176° F.).

When the copper was added to the milk *after* heating, the maximum stability of ascorbic acid was noted at the 80° C. (176° F.) exposure also. However, at the exposures of 80° C. (176° F.) and above the ascorbic acid remained comparatively stable for each amount of copper added, an observation not noted when the copper was added to the milk prior to heating.

The data showed that the development of oxidized flavor tended to follow the destruction of ascorbic acid. A marked decrease in the inten-

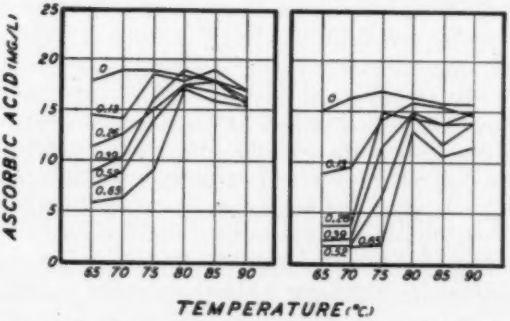



FIG. 5. The stability of ascorbic acid in milk when heated to various temperatures for ten minutes. The milk was contaminated with various amounts of copper (mg. per liter) *after* heating. Values at initial titration and after 24 hours storage.

sities of the oxidized flavor was noted when the milk was heated to 80° C. for ten minutes, the critical exposure noted in this experiment for the stability of ascorbic acid.

The time of adding copper with respect to the heat treatment was of importance also in the development of the oxidized flavor. When the copper was added to the milk after heating to 65 or 70° C. (149 or 158° F.) for ten minutes a distinct to a very strong oxidized flavor developed as contrasted to a slight oxidized flavor development when the copper was added before heating. At the 75 (167), 80 (176), and 85° C. (185° F.) exposures little difference was encountered in the intensities of the developed oxidized flavors, which required the presence of from 0.26 to 0.39 mg. of copper per liter for acceleration, regardless of its addition before or after heating. At the 90° C. (194° F.) exposure, however, the oxidized flavor was not noted in the milk contaminated with 0.52 or 0.65 mg. of copper per liter before heating, but was slight to distinct when similar amounts of copper were added after heating. This observation would seem to indicate that copper present in milk at the time of heating must have been combined in part or rendered unavailable for acceleration of the oxidation of the fatty constituents.

An important difference between the stability of ascorbic acid and the development of the oxidized flavor was noted. The ascorbic acid exhibited maximum stability at a definite exposure, 80° C. (176° F.) for ten minutes, whereas no specific exposure was noted to be effective under all conditions in preventing the oxidized flavor development. Several factors, such as the amount of copper added and the time of addition with respect to the heat treatment, were of importance in establishing an exposure which would inhibit the development of the oxidized flavor.

## DISCUSSION AND SUMMARY

Individual samples of milk from 20 cows were pasteurized for 30 minutes at 63° C. (145.4° F.) and at 75° C. (167° F.) with and without the addition of 0.13 mg. of copper per liter. The ascorbic acid was quite unstable in the milk processed at 63° C. (145.4° F.) with copper added following pasteurization. Such milk was very prone to the development of the oxidized flavor upon three days storage. Exceptions, however, were noted. The ascorbic acid of some cows' milk seemed to exhibit a natural stability lacking in other samples. The rapid disappearance of the reduced form of ascorbic acid was not always an assurance that oxidized flavor would develop, inasmuch as some samples were found having no ascorbic acid after three days storage, yet, although copper was present, oxidized flavor did not develop.

When the milk was pasteurized at 75° C. (167° F.) for 30 minutes, the ascorbic acid exhibited marked stability with no development of oxidized

flavor after three days storage regardless of the presence of 0.13 mg. of copper per liter.

Flash pasteurization of samples of milk from individual cows at temperatures ranging from 60–97° C. (140–206.6° F.) for fifteen seconds or less had varying effects upon the stability of ascorbic acid. The critical flash exposure minima for ascorbic acid stability were between 75 and 85° C. (167 and 185° F.); exposures below having slightly less stabilizing effect and exposures above having a more pronounced stabilizing effect than that existing naturally in the raw milk.

Samples of mixed milk from three cows were heated to 65 (149), 70 (158), 75 (167), 80 (176), 85 (185), and 90° C. (194° F.) for ten minutes with and without the addition of various amounts of copper. A temperature of 80° C. (176° F.) for 10 minutes seemed to be an exposure which caused marked changes in milk as far as factors affecting the stability of ascorbic acid and the development of the oxidized flavor were concerned. Whether copper was added before or after heating, the 80° C. (176° F.) exposure had the greatest stabilizing effect. When the copper was added to the milk *after* heat treatment a marked stability of the ascorbic acid was noted in the samples heated to 80° C. (176° F.) and above. However, when the copper was added *before* heat treatment this same stability was not observed. Furthermore, the addition of various amounts of copper before heat treatment seemed to have a greater destructive effect on the ascorbic acid, even at 80° C. (176° F.), than when the copper was added after heat treatment.

As has been shown previously (1) the time of adding copper with respect to the heat treatment was of importance in the development of the oxidized flavor. When the milk was exposed to 65° C. (149° F.) or to 70° C. (158° F.) for ten minutes and then treated with copper a distinct to a very strong oxidized flavor developed as contrasted to a slight oxidized flavor development when the copper was added before heating. At the 75 (167), 80 (176), and 85° C. (185° F.) exposures little difference was encountered in the intensities of the developed oxidized flavor, which required the presence of 0.26 to 0.39 mg. of copper per liter to develop a distinct oxidized flavor, regardless of its addition before or after heating. At 90° C. (194° F.), however, the oxidized flavor was not noted in the milk contaminated with 0.52 or 0.65 mg. of copper per liter before heating, but developed slightly when similar amounts of copper were added after heating.

Many observations on the flavor of milk treated at high temperatures in this and in other studies have shown a lessened tendency for the development of the oxidized flavor at temperatures at which the cooked flavor occurred. If distinct oxidized flavors developed up to a certain temperature of heat treatment they were generally "pure" oxidized after which

they usually become "pure" cooked. The similarity between the most effective temperatures in stabilizing the ascorbic acid and in retarding the development of the oxidized flavor found in these studies and the temperatures at which liberation of sulphides and cooked flavor occurred, as recently reported by Gould and Sommer (4), leads to the supposition that the formation of reducing substances at the higher temperatures, may play an important role both in the ascorbic acid titration values and in the development of the oxidized flavor.

## REFERENCES

(1) BROWN, W. CARSON, THURSTON, L. M., AND DUSTMAN, R. B. Oxidized flavor in milk. III. The time of copper contamination during production and processing, and aeration versus no aeration as related to oxidized flavor development. J. DAIRY SC., **19**: 753–760. 1936.

(2) CHILSON, W. H. What causes most common off flavors in market milk? Milk Plant Monthly, **24**: 11, 24–28, and **24**: 12, 30–34. 1935.

(3) DAHLE, C. D., AND PALMER, L. S. The oxidized flavor in milk from the individual cow. Pa. Agr. Exp. Sta. Bull. 347. 1937.

(4) GOULD, I. A. JR., AND SOMMER, H. H. Effect of heat on milk with especial reference to the cooked flavor. Mich. Agr. Exp. Sta. Tech. Bull. 164. 1939.

(5) GREENBANK, GEORGE R. Control of the oxidized flavor in milk. Proc. 29th Ann. Conv. Int. Assoc. Milk Dealers, Lab. Sect. p. 101. 1936.

(6) GUTHRIE, E. S., AND BRUECKNER, H. J. The cow as a source of "oxidized" flavors of milk. N. Y. (Cornell) Agr. Exp. Sta. Bull. 606. 1933.

(7) HAND, DAVID B., GUTHRIE, E. S., AND SHARP, PAUL F. Effect of oxygen, light and lactoflavin on the oxidation of vitamin C in milk. Science, **87**: 2263, 439–441. 1938.

(8) KENDE, S. Untersuchungen über, 'olig-talgige,' 'schmirgelige,' Veränderungen der Milch. Milchw. Forsch., **13**: 111–143. 1932.

(9) KING, C. G., AND WAUGH, W. A. The effect of pasteurization upon the vitamin C content of milk. J. DAIRY SC., **17**: 489–495. 1934.

(10) SHARP, PAUL F., TROUT, G. MALCOLM, AND GUTHRIE, E. S. Vitamin C, copper, and the oxidized flavor of milk. Tenth Ann. Rept. N. Y. State Assoc. of Dairy and Milk Inspectors, pp. 153–64. 1936.

(11) SHARP, P. F. Rapid method for the quantitative determination of reduced ascorbic acid in milk. J. DAIRY SC., **21**: 85–87. 1938.

(12) THURSTON, L. M. Oxidation in relation to off flavors in milk and certain milk products. Food Research, **2**: 255–272. 1937.

(13) WHITNAH, C. H., RIDDELL, W. H., AND CAULFIELD, W. J. The influence of storage, pasteurization and contamination with metals on the stability of vitamin C in milk. J. DAIRY SC., **19**: 373–383. 1936.

# THE EFFECT OF FEEDING PEA VINE SILAGE ON THE CAROTENE AND VITAMIN A CONTENT OF MILK[1,2]

B. CONNOR JOHNSON AND W. H. PETERSON

Many farmers in pea canning areas have found that silage made from pea vines is a satisfactory feed for dairy cattle. A preliminary report was published last year (1) indicating that well-made pea vine silage may prove a valuable feeding material for improving the vitamin A potency of winter milk. A cooperative experiment was carried out in the winter of 1938–39 with Warren G. Clark of the Central Wisconsin Canneries at Beaver Dam, on the feeding of pea vine silage to his dairy herd. The experiment was primarily to determine the efficiency of this type of silage in the production of milk of a high carotene and vitamin A content. A group of Holstein cows was fed this silage, while at the same time other cows of the same herd were fed the same dry ration without the added silage. Carotene determinations were made on the silage, and carotene

TABLE 1

*Analysis of pea vine silage*

| No. | Date | Treatment | Appearance and odor | Dry matter | pH | Carotene |
|---|---|---|---|---|---|---|
| | | **Pea Vine Silages from the Warren G. Clark Farm** | | | | |
| | | | | *per cent* | | |
| 2 | December 16, 1938 | $H_3PO_4$ | Good | 26.0 | 4.2 | 189 |
| 3 | January 9, 1939 | " | " | 21.3 | 4.4 | 98 |
| 4 | February 6 | " | " | 21.6 | 4.3 | 114 |
| 6 | March 21 | " | " | 26.4 | 4.8 | 117 |
| 7 | April 17 | " | " | 29.0 | 3.9 | 130 |
| | | **Pea Vine Silages from Other Sources*** | | | | |
| 1 | November 16, 1938 | $H_3PO_4$ | Good | 23.5 | 3.10 | 163 |
| 2 | " 15 | A.I.V. | " | 19.7 | 4.8 | 232 |
| 3 | March 20, 1939 | " | " | 31.6 | 3.65 | 187 |
| 4 | December 16, 1938 | None | " | 20.0 | 4.8 | 328 |
| 5 | April 17, 1939 | " | Poor odor | 23.9 | 5.46 | 170 |
| 6 | February 6, 1939 | " | Good | 21.8 | 4.5 | 172 |
| 7 | January 12 | " | Fair | 20.2 | 5.1 | 50 |
| 8 | " 12 | " | Poor | 18.8 | 5.1 | 7 |
| 9 | December 16, 1938 | Stack, none | Good | 27.7 | 4.15 | 186 |
| 10 | " 24 | " " | " | 23.0 | 4.32 | 184 |
| 11 | March 20, 1939 | " " | " | 23.5 | 4.07 | 145 |
| 12 | December 16, 1938 | " " | " | 30.1 | 4.45 | 120 |

* These are silo silages, unless otherwise stated.

Received for publication October 28, 1939.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, University of Wisconsin, Madison.

[2] The authors are indebted to Warren G. Clark and Berger Sandstrom for their cooperation in planning and carrying out the feeding work of this paper.

and vitamin A determinations on the milk at regular intervals. Milk production records were also kept during the experiment.

This pea vine silage was prepared with 55 per cent phosphoric acid added at the rate of one gallon per ton. The acid was diluted 1 to 4 and put on the forage in layers, that is, pea vines were filled in for about one foot, diluted acid was poured over it; then another foot of pea vines was added and followed by acid, and in this way the silo was filled. This uneven addition of acid may explain the variations in carotene content. The carotene in the silage was determined by the method of Hegsted, Porter and Peterson (2). The results of the silage analysis are given in table 1. For comparative purposes, analysis of samples of pea vine silage of various types from other parts of Wisconsin are included in this table.

Twenty-two cows were maintained on a ration consisting of 15 pounds of good quality alfalfa hay, 10 pounds dry corn stalks, 13 pounds corn and cob meal and soybean hay (equal parts by weight) and 25 pounds pea vine silage per cow per day, for the 17 weeks of the experiment. After several weeks on this ration, three cows were changed to a ration in which

TABLE 2

*Analysis of milks from cows fed pea vine silage*

| Date | Carotene* | Vitamin A* | Total potency* | Ration** |
|---|---|---|---|---|
| **Herd cows** | | | | |
| January 3, 1939 | 3.8 | 11.1 | 13.0 | Pea vine silage |
| " 9 | 3.1 | 11.1 | 12.7 | " " " |
| " 23 | 2.7 | 11.0 | 12.4 | " " " |
| February 6 | 3.2 | 13.6 | 15.2 | " " " |
| " 14 | 3.3 | 13.2 | 14.8 | " " " |
| March 15 | 2.9 | 10.7 | 12.2 | " " " |
| " 21 | 3.0 | 9.7 | 11.2 | " " " |
| April 18 | 2.5 | 9.1 | 10.4 | " " " |
| **Experimental cows** | | | | |
| December 20, 1939 | 3.7 | 10.6 | 12.5 | Pea vine silage |
| January 3, 1939 | 3.7 | 11.5 | 13.4 | Silage omitted |
| " 9 | 3.4 | 10.6 | 12.3 | " " |
| " 23 | 2.6 | 9.0 | 10.3 | " " |
| February 6 | 2.5 | 8.7 | 10.0 | " " |
| " 14 | 2.9 | 9.0 | 10.5 | Silage begun again |
| " 28 | 3.7 | 10.2 | 12.1 | " continued |
| March 15 | 5.7 | 9.6 | 12.4 | " " |
| " 21 | 2.5 | 10.7 | 12.0 | " " |
| April 18 | 3.1 | 10.3 | 11.9 | " " |
| **Milk from other herds on pea vine silage** | | | | |
| | | | | Source |
| December 16, 1938 | 7.1 | 12.4 | 15.9 | Columbus |
| February 17, 1939 | 5.3 | 9.1 | 11.8 | " |
| January 4, 1939 | 4.3 | 9.1 | 11.2 | Rockmarsh |

* Expressed in micrograms per gram butterfat.

** See Table 3 for complete ration.

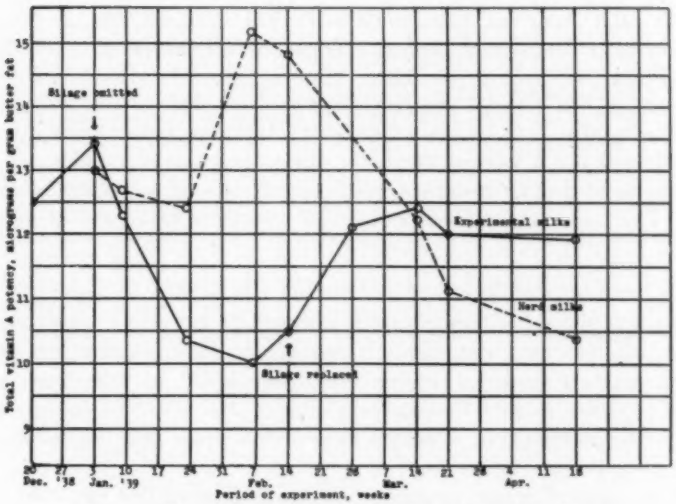



FIG. 1. Vitamin potency of herd and experimental milks.

the silage was replaced by alfalfa hay on an equivalent dry matter basis. They were kept on this dry ration for 6 weeks, at which time the silage was again placed in the ration.

TABLE 3

*Milk production of experimental cows*

| Date | Pounds of milk produced | Ration per cow |
|---|---|---|
| *Fore Period. Silage* | | |
| December 20, 1938 | 55.7 | Hay, alfalfa 15 lbs., pea vine silage, 25 lbs., corn stalks 10 lbs., corn and cob meal and soy bean hay 13 lbs. |
| December 27 | 53.0 | Same |
| January 3, 1939 | 55.0 | " |
| *Test Period. No silage* | | |
| January 6, 1939 | 49.9 | Same as fore period except pea silage replaced with alfalfa hay. |
| January 9 | 44.3 | Same |
| January 17 | 45.0 | " |
| January 25 | 46.5 | " |
| February 7 | 43.0 | " |
| February 15 | 41.5 | " |
| *After Period. Silage* | | |
| February 22 | 40.8 | Same as fore period. |
| February 26 | 40.5 | " " " " |
| March 14 | 42.0 | " " " " * |
| March 21 | 39.3 | " " " " * |
| April 17 | 36.7 | " " " " * |

* Pea vine-sudan hay silage instead of pea vine silage.

The milk from both groups of cows was analyzed for carotene and vitamin A by the method of Olson, Hegsted and Peterson (3), at intervals throughout the experiment. The results are given in table 2 and plotted on figure 1. In table 2 are also given for comparison, the results from milks from other cows on pea vine silage. In figure 1 the curves represent the total vitamin A value of the milk. In making this calculation, carotene is taken as having a potency one-half that of vitamin A, weight for weight (4). The milk production for the three experimental cows is given in table 3.

## DISCUSSION

Pea vine silage has been reported as worth 91 per cent as much as good corn silage for dairy cows (5). It was also pointed out that less protein supplements are needed when pea vine silage is fed, as this silage is considerably richer in protein than corn silage. Morrison (6) has given the analysis for nutrients of a sample of pea vine silage and has pointed out its value in live stock feeding.

From figure 1 it can be seen that the feeding of this pea vine silage at 25 pounds a day maintained the vitamin A and carotene at a high level. When the silage was omitted from the ration, the vitamin A value of the milk quickly decreased. On replacement of silage in the ration of the cows that had been on dry feed for six weeks, the vitamin A value of their milk rose again to approximately its former level, and a level equal to that of milk produced by the cows that had received silage throughout the experiment. This shows definitely that the silage was responsible for the high vitamin A value of this milk. Comparison shows these values to be much higher than the average values of winter market milks in this state. These were found by Dornbush *et al.* (7) for January 1939 to April 1939 to average approximately 8.8 micrograms per gram butter fat.

Also from table 3 the rate of decrease of milk production is found to be more rapid during the period when the cows did not receive silage. There was a decrease of about 0.4 pound per week in the fore period (two weeks), 2.2 pounds per week in the experimental period (six weeks) and 0.6 pound per week in the after period (eight weeks).

## SUMMARY

Cows fed a good quality of pea vine silage produced a milk higher in carotene and vitamin A than when fed a dry ration. They also maintained milk production more satisfactorily on the silage than on the dry ration. It should not be concluded from these results, of course, that pea vine silage is always superior to alfalfa hay. Because of variations in the quality of both silage and hay, a general conclusion could not be drawn until after extended comparisons had been made.

## REFERENCES

(1) PETERSON, W. H. Pea vine silage and quality milk. Canning Trade, 60: No. 45, 7. 1938.

(2) HEGSTED, D. M., PORTER, J. W., AND PETERSON, W. H. Determination of carotene in silage, an improved method. Ind. Eng. Chem., Anal. Ed., 11: 256. 1939.

(3) OLSON, F. R., HEGSTED, D. M., AND PETERSON, W. H. Determination of carotene and vitamin A in milk. J. DAIRY SC., 22: 63. 1939.

(4) MEAD, T. H., UNDERHILL, S. W. F., AND COWARD, K. H. Crystalline esters of vitamin A. II. Biological Potency. Biochem. J., 33: 594. 1939.

(5) WISCONSIN BULLETIN. Pea vine silage compared with corn silage. 425: 120–121. 1933.

(6) MORRISON, F. B. The feeding value of canning factory silages. Canner, 79: No. 10, 14. 1934.

(7) DORNBUSH, A. C., PETERSON, W. H., AND OLSON, F. R. The carotene and vitamin A contents of Wisconsin market milks. J. Am. Med. Assoc. In press.

# THE RELATION OF IODINE AND PEROXIDE NUMBERS TO OXIDIZED FLAVOR OF MILK*

D. H. NELSON AND C. D. DAHLE

*Dairy Department, Pennsylvania State College*

Observations on the iodine and peroxide numbers of milk fat have led to conflicting reports as to whether the milk fat itself or the fat globule "membrane" lecithin is the substance oxidized to give this flavor defect. Brown *et al.* (1) found no change in the iodine number of the fat when a copper-induced flavor had developed. Ross (2) found no relationship between the iodine number and the oxidized flavor of ice cream. Several studies (3, 4, 5, 6, 7) of the oxidation of butter and pure milk fat indicate no significant changes in any of the fat constants until the characteristic flavor and the fading of the natural color give unmistakable evidence of an advanced state of deterioration. It has also been reported (8, 9) that the oxidized flavor appears before the end of the induction period of the fat, that is, in the early stages of deterioration. Briggs (3) has shown that peroxides, formed in the process of fat oxidation, do not interfere with or change the iodine value. Therefore a decrease in iodine number coincident with the development of the off-flavor would not necessarily be expected although the milk fat itself were the substance being oxidized to give the flavor. However, Kende (10) and Dahle and Palmer (11) observed a lower iodine number in the fat of spontaneously oxidized samples than in the heated control samples, suggesting that the milk fat itself had been oxidized.

Dahle and Palmer held their samples "until the degree of oxidized flavor did not show any increase," possibly allowing the fat to reach the end of its induction period before the iodine number was determined. Furthermore, their unpublished data show that the milk fat obtained from the milk in all cases was practically colorless even when churned direct from the cow. This suggests that a very pale colored susceptible fat might be associated with the development of the oxidized flavor and with the stability of the fat.

Swanson and Sommer (13) determined the iodine number of the fat and the phospholipid fraction of oxidized flavored milk (copper induced) and found no change in the iodine number of the fat, but a lowering in the iodine number of the phospholipid fraction, indicating that the fat is not affected when an oxidized flavor occurs.

Stebnitz and Sommer (7) found no relation between the carotene content as indicated by the color, and the stability of the fat. This is in agreement with certain observations (11, 12) that the oxidized flavor bears no

Received for publication November 13, 1939.

* Authorized for publication on November 8, 1939 as paper No. 931, in the Journal Series of Pennsylvania Agricultural Experiment Station.

relation to the breed of cow producing the milk. Garrett *et al.* (14) observed that there was a close relation between the per cent fat and the yellow color of the milk, and that a high carotene content is coincidental to and helps preserve a good flavor. On the other hand Beck *et al.* (15) concluded that milk below the breed average in color intensity appeared to be related to oxidized flavor. The beneficial effects obtained by Anderson *et al.* (16) in feeding pure carotene to prevent the development of oxidized flavor might be interpreted to support the findings of Beck *et al.* Although there may be no correlation between the color and stability of milk fat or between the color and oxidized flavor of the milk, there is some evidence that a subnormal color is associated with the development of oxidized flavor.

## EXPERIMENTAL

In these experiments milk was obtained from cows known to produce the spontaneous oxidized flavor. Care to avoid possible metal contamination and exposure to light was exercised. Part of the milk was centrifugally separated and the cream standardized to 20 per cent fat by adding some of the original whole milk. The remaining milk and the cream were each divided into three portions, one being unheated, one heated to 80° C. for 10 minutes to inhibit the oxidized flavor, and the other heated to 62° C. for 30 minutes. The heated samples were cooled and stored in the dark at 4.5° C. until examined and analyzed.

Samples of milk and cream known to be resistant to spontaneous flavor were prepared in exactly the same manner except that copper was added to the lots heated at 62° C., after the pasteurizing was completed.

The samples were examined for flavor when fresh and at 24-hour intervals until a pronounced oxidized flavor had developed and again at the time analysis of the fat was made. The iodine and peroxide numbers were determined on the fresh samples and again when a pronounced oxidized flavor had developed and at various intervals during storage. The fat was obtained by churning sufficient milk or cream into butter which was then heated to 60° C. and transferred to a 50 ml. pyrex centrifuge tube, and centrifuged at 3500 r.p.m. for five minutes. The dry, pure, milk fat was removed and the iodine number determined by the Hanus Method and the peroxide number by the method described by Greenbank and Holm (17).

Typical results of the experiments with copper-induced flavor appear in table 1 and indicate no significant change in the iodine number or the peroxide number even when a very strong oxidized flavor had developed. The samples stored for six weeks showed some protein decomposition. A strong oxidized flavor always appeared in the pure milk fat after storage although to the unaided eye there was no apparent fading of the natural color of the fat. Similar findings in regard to the iodine number have been reported before.

TABLE 1

*Relation of iodine and peroxide numbers to copper-induced oxidized flavor*

| Samples | Fresh | | | 2 days | | | 5 days | | | 3 weeks | | | 6 weeks | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number |
| (1) Control milk | – | 32.2* | 0.0 | – | 32.6 | 0.0 | – | 32.5 | 0.0 | | | | | | |
| (2) Milk + 2 p.p.m. copper | – | 32.2 | 0.0 | ++ | 32.7 | 0.0 | +++ | 32.6 | 0.0 | | | | | | |
| (3) Control cream | – | 32.4 | 0.0 | – | | | – | 32.5 | 0.0 | – | 31.9 | 0.0 | – | 31.8 | 0.0 |
| (4) Cream + 6 p.p.m. copper | – | 32.6 | 0.0 | + | | | ++ ++ | 32.5 | 0.0 | ++ ++ | 32.1 | 0.0 | ++ ++ | 32.0 | 0.6 |

* Iodine numbers in tables 1 and 3 are higher than they are in table 2. The milk used in table 1 came from another herd as the milk from the station herd failed to produce the spontaneous oxidized flavor.

Since four experiments using spontaneous milk gave the same results, only one experiment is included in table 2. No significant change in either the iodine or peroxide number occurred in samples having an oxidized flavor. The milks showed some protein decomposition after the long storage period at the low temperature.

In holding these samples for such a long period of time a phenomenon occurred which was quite puzzling at the time. The sample heated to 80° C. showed no oxidized flavor but a cooked flavor as has always been the case. Later in the storage period the cooked flavor disappeared and finally after three weeks in storage a strong oxidized flavor occurred. This was contrary to previous findings although previously samples showing the elimination of the oxidized flavor with high temperatures were always discarded after approximately 72 hours. It has been assumed that the high temperature of heating destroyed an enzyme that was responsible for the oxidized flavor.

In view of the work of Gould and Sommer (19), and Josephson and Doan (20) one may attempt to explain this phenomenon on the basis that the high heat produced reducing substances which prevented the development of the oxidized flavor. On long holding at low temperatures the reducing substance became oxidized and the fat or fatty material in turn became oxidized giving rise to the oxidized flavor in this spontaneous milk.

When oxidized flavor occurred in the milk or cream this flavor always carried over in the butter and the pure fat, yet the fat did not change in color. Unfortunately, spontaneous milk having initially a very pale-colored fat was not available for these experiments.

This lack of fading of the natural color indicates, as will be shown later, that the fat had not reached the end of its induction period. This is believed to be a significant factor in explaining the discrepancy between the results of these experiments and the results reported by others showing the relation of iodine number to spontaneous oxidized flavor. While Dahle and Palmer (11) fail to mention the color of the fat in their experiments their unpublished results show that the milk fat obtained from the milk was practically colorless, even direct from the cow. Possibly this pale-colored fat had a very short induction period. The significance of this is emphasized by the experiments to follow.

The fact that the peroxide number of the fat was always zero, even when the fat had a strong oxidized flavor, suggested that the peroxides might have been removed during the isolation of the fat. If such were the case the flavor of the fat could be explained by assuming the flavor substance to be absorbed by the fat and insoluble in water. The following experiment represents many attempts to remove the peroxides by washing the fat with hot water.

Dry, fresh milk fat was obtained from sweet, one-day-old cream that was known to be free from spontaneous oxidized flavor and to be free from any

TABLE 2

*Relation of iodine and peroxide numbers to spontaneous oxidized flavor*

| Treatment of sample | Fresh | | | 2 days | | | 5 days | | | 3 weeks | | | 6 weeks | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number | Flavor | Iodine number | Peroxide number |
| Milk, unheated | – | 28.0 | 0.0 | + | | | ++ | 28.1 | 0.0 | +++ | 27.8 | 0.0 | Bitter | 27.9 | 0.0 |
| Milk, heated 62° C. 30 min. | – | 27.7 | 0.0 | + | | | +++ | 28.0 | 0.0 | +++ | 27.9 | 0.0 | +++ | 27.7 | 0.0 |
| Milk, heated 80° C. 10 min. | Cooked | 27.8 | 0.0 | Cooked | | | Cooked | 27.8 | 0.0 | ++ | 27.8 | 0.0 | ++ | 27.6 | 0.0* |
| Cream, unheated | – | 28.4 | 0.0 | – | | | – | 28.0 | 0.0 | + | 27.8 | 0.0 | – | 28.0 | 0.0 |
| Cream, heated 62° C. 30 min. | – | 28.1 | 0.0 | – | | | + | 28.2 | 0.0 | + | 28.4 | 0.0 | + | 28.0 | 0.4 |
| Cream, heated 80° C. 10 min. | – | 28.0 | 0.0 | – | | | – | 28.5 | 0.0 | – | 28.2 | 0.0 | – | 27.8 | 0.0 |

* This sample developed an oxidized flavor after 3 weeks in storage although it had a cooked flavor prior to that time.

metal contamination. This fresh cream was churned in a glass churn, the butter transferred to a separatory funnel and washed with hot (71° C.) water until the wash water was clear, which required three or four washings with a volume of water equal to the volume of butter each time. The hot fat was centrifuged for five minutes at 3500 r.p.m. The fat obtained was then placed in a carefully cleaned and stoppered flask, and incubated in the dark at 60° C.

The fat was examined at intervals for flavor, peroxide number, iodine number and for any change in color noticeable without the aid of color standards. At certain stages of oxidation, as indicated by the peroxide number, a portion of the fat was transferred to a separatory funnel and washed three times, each time using a volume of clean hot (65° C.) water slightly greater than the volume of the fat. The fat was centrifuged for five minutes at 3500 r.p.m. and the peroxide number and flavor determined.

TABLE 3

*Flavor and peroxide numbers of washed oxidized fat*

| Days in dark at 60° C. | Color | Iodine number | Flavor | Peroxide number | Rewashed fat | |
|---|---|---|---|---|---|---|
| | | | | | Peroxide number | Flavor |
| 0 | Yellow | 33.6 | − | 0.0 | | |
| 4 | Yellow | | ± | 0.0 | | |
| 7 | Yellow | 33.3 | + | 0.3 | | |
| 10 | Yellow | | ++ | 1.8 | 1.7 | ++ |
| 15 | Pale yellow | | ++ | 8.0 | 8.0 | ++ |
| 25 | White | 31.3 | ++ | 46.0 | 45.5 | ++ |

The results in table 3 indicate that neither the peroxides nor the flavored substance are removed by washing the fat with hot water. Therefore the low peroxide number in tables 1 and 2 of fat isolated from milk or cream having a strong oxidized flavor cannot be due to the removal of peroxides during the isolation of the fat. Furthermore, in the autoxidation of pure fresh milk fat the authors have observed the appearance of the oxidized flavor as soon as, or slightly before the appearance of any peroxide number. It, therefore, seems that the determination of the peroxide number is not a sufficiently sensitive method to detect the extremely small concentrations of peroxides which may be produced during the formation of the flavored substance. This undoubtedly explains why Koenig (18) found no suitable correlation between the peroxide number and the flavor of butter.

The observation that the oxidized flavor appears before the end of the induction period has been observed by others (8, 9).

In the autoxidation of pure milk fat the authors noted that the fading of the natural color of the fat occurred with the beginning of the rapid increase in peroxide number, that is, at the end of the induction period. This is indicated in table 3 which also shows that the significant decrease in the

iodine number is more closely associated with fading of the natural color and the end of the induction period than it is with the appearance of the oxidized flavor. This agrees with the report of Briggs (3) who found no significant change in either the peroxide number or the iodine number during the induction period.

Since the oxidized flavor appears early in the induction period, and since the change in iodine number is associated with fading of the natural color and the end of the induction period, it may be possible that a very pale-colored fat would show such a short induction period that the decrease in iodine number would appear approximately at the same time that the oxidized flavor appeared. In such a case, this would explain why Dahle and Palmer (11) using spontaneous milk with a very pale colored fat observed a decrease in iodine number, while the above results on spontaneous milk with a fat having an appreciable color and induction period did not show a decrease in iodine number with the appearance of the oxidized flavor.

## SUMMARY

That the iodine number of milk fat does not decrease with the early development of the oxidized flavor, agrees with the majority of similar reports. This finding, however, is in conflict with certain investigations on spontaneous milk in which the iodine number was found to decrease.

It was observed that milk fat usually had a peroxide number of zero when freshly isolated, even from milk or cream having a strong oxidized flavor, which was carried over into the pure fat. Evidence is presented indicating that it is not due to removal of the peroxide during the isolation of the fat. It is also shown that the peroxide number and iodine number do not change materially until the color of the fat is affected.

## REFERENCES

(1) Brown, W. C., Dustman, R. B., and Thurston, L. M. Oxidized flavor in milk. V. The effect of metal-developed oxidized flavor on the iodine number of the milk fat. J. Dairy Sc., 20: 599. 1937.

(2) Ross, O. E. Why does a tallowy flavor develop in strawberry ice cream? Ice Cream Trade J. 29: 7, 23. 1933.

(3) Briggs, L. H. The autoxidation of butterfat. II. Comparison of tests for detecting oxidation changes. J. Dairy Res., 3: 70. 1931.

(4) Browne, C. A. A contribution to the chemistry of butterfat. J. Am. Chem. Soc., 21: 612. 1899.

(5) Hunziker, O. F., and Hosman, D. F. Tallowy butter, its causes and prevention. J. Dairy Sc., 1: 320. 1917.

(6) Overman, O. R., Garrett, O. F., and Ruehe, H. A. Studies on the keeping quality of butter in cold storage. Ill. Sta. Bull. 446. 1938.

(7) Stebnitz, V. C., and Sommer, H. H. The oxidation of butterfat. I. *The oxidation of butter fat.* II. The composition of fat in relation to its susceptibility toward oxidation. J. Dairy Sc., 20: 5, 265. 1937.

(8) HENDERSON, J. L., AND ROADHOUSE, C. L. Factors influencing the initial induction period in the oxidation of milk fat. J. DAIRY SC., 17: 321. 1934.

(9) STEBNITZ, V. C., AND SOMMER, H. H. The oxidation of butterfat. I. The catalytic effect of light. J. DAIRY SC., 20: 181. 1937.

(10) KENDE, S. Untersuchungen über 'olig-tagige,' 'schmirgelige,' Veränderungen der Milch. Milchw. Forsch., 13: 111–143. 1932.

(11) DAHLE, C. D., AND PALMER, L. S. The oxidized flavor in milk from the individual cow. Penna. Agr. Exp. Sta. Bull. 347. 1937.

(12) GUTHRIE, E. S., AND BRUECKNER, H. J. The cow as a source of "oxidized" flavors of milk. Cornell Sta. Bull. 606. 1933.

(13) SWANSON, A. M., AND SOMMER, H. H. Oxidized flavor in milk traced to fat related substances. Wisc. Agr. Exp. Sta. Bull. 442: 75. 1938.

(14) GARRETT, L. F., TUCKER, H. H., AND BUTTON, F. C. Relation of color and ascorbic acid to flavor in milk from individual cows. J. DAIRY SC., 21: 121. 1938.

(15) BECK, G. H., WHITNAH, C. H., AND MARTIN, W. H. Relation of vitamin C, lecithin and carotene of milk to the development of oxidized flavor. J. DAIRY SC., 22: 17. 1939.

(16) ANDERSON, J. A., WILSON, L. T., AND HARDENBERGH, J. H. The causes of off-flavors in milk; the facts and a theory. Ann. Conv. Intern. Assoc. M. Dealers. Lab. Sect. 177. 1937.

(17) GREENBANK, G. R., AND HOLM, G. E. Antioxidants for fats and oils. Ind. Eng. Chem., 26: 243. 1934.

(18) KOENIG, V. L. Stabilization of butter against oxidized flavors. Nat'l Butter and Cheese J., 28: 15, 26. 1937.

(19) GOULD, I. A., AND SOMMER, H. H. Effect of heat on milk with especial reference to the cooked flavor. Mich. Tech. Bull. 164. 1939.

(20) JOSEPHSON, D. V., AND DOAN, F. J. Paper presented before East. Sect. Am. Dairy Sc. Assoc., Springfield, Mass. September, 1939.

# A YOLK–BUFFER PABULUM FOR THE PRESERVATION OF BULL SEMEN[1]

PAUL H. PHILLIPS AND HENRY A. LARDY

*Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wisconsin*

The difficulty of consistently preserving semen has been a serious obstacle in the progress of artificial insemination. In many cases low breeding efficiency has accompanied artificial insemination due to improper or faulty preservation and storage of the semen sample. This is particularly true when it was desired to use a stored sample over a considerable period of time.

In the studies herein reported we attempted to determine certain conditions under which bull semen might be environed to maintain its potency. Our object was threefold: (a) to develop a suitable nutrient medium; (b) to obtain a nutrient solution that would maintain potency for a period of several days, thus eliminating the daily use of the bull; and (c) to develop a procedure which was applicable under practical conditions.

A study of the literature indicates that buffered glucose solutions have been tried by Baker (2), Anderson (1), Mclovanov (7), Hyme (5), Walton (10), Winters *et al.* (11), and others. Ivanov (6) and Bernshstein (3) have presented some evidence to indicate that glucose was not the energy metabolite used by spermatozoa. No clear-cut evidence was available to indicate the most favorable storage pH for semen. Hatziolos (4) reported the average pH of 54 samples of bull semen to be 6.89. Shergin's data (9) indicate that the semen of most species is slightly alkaline. He gives a pH value of 6.74 for the bull. Anderson (1) uses a dilutor for ram semen buffered at pH 7.6.

## EXPERIMENTAL

In order to keep these studies on a practical basis it seemed wise to first test naturally-occurring material. Since the egg is the counterpart of spermatozoa it seemed to be a likely starting material. Because of the buffering action of proteins, fresh egg white was used both with and without added glucose. This medium did little if any better than the control sample of raw semen stored under ideal physical conditions.

Next a mixture of fresh egg yolk (hen) and a phosphate buffer was tried. The buffer was made up to a pH of 7.0 from M/15 potassium and

Received for publication November 13, 1939.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Wisconsin Alumni Research Foundation. We wish to acknowledge the fine cooperation given to us by Drs. H. D. Doudna, William Thomson, Walter Wisnicky, and the Dairy Husbandry and Poultry Husbandry Departments.

sodium phosphate solutions and mixed with an equal volume of fresh raw egg yolk. The semen was diluted 4:1 with this mixture. The results were extremely satisfactory. A repetition of the experiment was not so encouraging. A study of the pH of the egg yolks in use showed a wide variation in pH (6.0–6.53). Yolk-buffer mixtures buffered at pH 6.6–6.65 again gave excellent results. These experiments indicated that bull semen could be stored in buffered egg yolk. Egg yolk alone was less effective than buffered egg yolk.

It appeared certain that controlled pH and a buffer system were necessary for the successful storage of semen for long periods. Phosphate buffers of various strengths, M/5, M/10, M/12, and M/15 were tried. Buffering with a stronger solution than M/12 phosphate was found to be detrimental. A series of studies was then made to determine the optimum pH for storage. Semen from ten bulls was stored in yolk-buffer solutions ranging in pH from 6.0–7.5. The optimum pH for storage was found to be 6.75 and the narrow pH range of 6.7–6.8 gave the best preservation of motility. The results are shown in figure 1. If the pH is skewed slightly

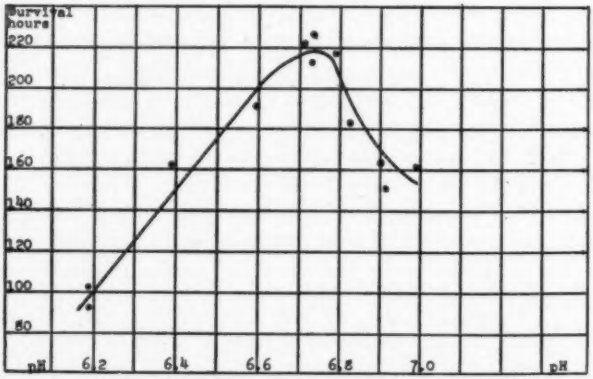



FIG. 1. A representative curve showing the effect of pH of the yolk-buffer upon sperm survival.

to the alkaline side to 6.8–6.9, a low grade (+) motility can be maintained for a longer period than with a pH of 6.75. However, active motility of the order of +++ falls off much more quickly at pH 6.9. Considerable acidity develops during storage. At the end stage the pH is usually near 6.0–6.1. It is possible that the higher pH delays the development of acid in the final stages and thereby increases sperm survival. That this is not entirely the case is indicated by the fact that fresh buffer added from time to time to correct and maintain the proper pH does not prolong survival.

The most favorable ratio of semen: yolk-buffer was next determined. It was found that excellent results were obtained with ratios up to 1:5.

The latter seemed to be the upper practical limit and the best routine results were obtained with a ratio of 1 : 3 or 4. Optimum storage temperatures indicated maximum storage in the temperature range from 5–10° C. Temperatures outside of this range were definitely inferior.

In connection with these studies numerous other substances and solutions have been tried. A dried pork liver suspension, dried liver extract solution, bovine blood serum and blood plasma, buffered egg yolk fat and materials collected from the testes and accessory glands of the bull were without benefit if not actually harmful to the sperm. Table 1 indicates that some success was obtained with dried egg yolk-buffer and boiled muscle extract.

TABLE 1

*The comparison of various media with egg yolk-buffer as a pabulum for sperm survival*

| Media | Motility and survival time in hours | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 48 | 72 | 96 | 120 | 150 | 200 | 225 | 300 |
| Buffered glucose | dead | | | | | | | |
| Buffered lecithin (egg) | + | dead | | | | | | |
| Evaporated milk (irrad.) | ++ | dead | | | | | | |
| Chick brain substance | ++ | + | dead | | | | | |
| Boiled muscle extract | +++ | +++ | ++ | dead | | | | |
| Buffered dry egg yolk | +++ | +++ | ++ | dead | | | | |
| Buffered egg yolk | +++++ | +++++ | +++++ | +++++ | +++++ | +++ | ++ | + |

These studies thus showed: (a) that egg-yolk furnished the necessary metabolite, or metabolites required by the spermatozoa of the bull, (b) that a buffered solution in a rather narrow restricted pH was necessary, and (c) that the proper combination of these two factors was highly satisfactory for the preservation of bull semen. With these results to guide us, the following procedure was developed and previously published in part (8).

The buffer solution was made up with 0.2 gram of $KH_2PO_4$ and 2.0 grams of $Na_2HPO_4 \cdot 12H_2O$ per 100 cc. of distilled and sterilized $H_2O$. This buffer can be kept in the refrigerator for short periods but works best when made up fresh. Equal amounts of the buffer and fresh egg yolk are then mixed. The proportion of buffer in this preparation is designed to yield a mixture whose final pH is 6.75. It should be checked before use and if need be adjusted. In 90 per cent of the cases the pH will be 6.75. Adjustment of pH is made with sterile solutions of either the monobasic potassium, or dibasic sodium salt as required. Yolk-buffer

mixtures with a pH lying between 6.7–6.8 will be found to be satisfactory. The yolk-buffer preparation is now ready for use.

Freshly collected semen (the second ejaculate is often preferable where the bull is not used regularly and often enough) was then combined with the yolk-buffer in the proportion of 1:3 or 1:4. The yolk-buffer semen sample was then stored in stoppered containers kept in the refrigerator at a temperature of 10° C. No undue care has been found necessary in the cooling or warming of the sample. One can go from room temperature to 10° C. and back to room temperature without serious damage.

Semen thus preserved maintains its original activity with remarkable consistency. A high grade, very active semen will maintain a high degree of activity for 100 hours and more. Semen from the bulls used in these tests maintained excellent activity up to 150 hours. Thereafter there was a gradual loss of motility during the next 100 hours. It was not at all uncommon for some activity to last beyond 300 hours.

Semen suspended in yolk-buffer has been successfully shipped from British Columbia to the middle west and from coast to coast.

In much of this experimental work we have relied upon the microscope and sperm motility for the detection of semen quality. It appears that, within certain limits, a yolk-buffer-semen sample which shows vigorous activity under the microscope will usually be found to be potent. Table 2 indicates that yolk-buffered semen is potent for a considerable time.

TABLE 2

*Summary of breeding records with yolk buffered semen on cows bred for sixty-three or more days*

| Y.B. semen age (hrs.) | No. of cows bred | Total services | No. diagnosed pregnant | Total* No. apparently pregnant | No. poor breeders** |
|---|---|---|---|---|---|
| 1–12 | 28 | 33 | 9 | 20 | 14 |
| 12–24 | 11 | 12 | 1 | 5 | 3 |
| 24–36 | 58 | 68 | 17 | 35 | 21 |
| 36–48 | 2 | 2 | 1 | 2 | 1 |
| 48–60 | 14 | 15 | 5 | 5 | 6 |
| 60–72 | 2 | 2 | 0 | 1 | 1 |
| 72–84 | 2 | 4 | 1 | 1 | 2 |
| 84–96 | 0 | 0 | 0 | 0 | — |
| 96–108 | 8 | 8 | 1 | 2 | 4 |
| 124 | 1 | 1 | 1 | 1 | 0 |
| 151 | 1 | 1 | 1 | 1 | 0 |
| 180 | 1 | 1 | 1 | 1 | 0 |
| 196 | 1 | 1 | 0 | 0 | 1*** |

* This figure includes the cows diagnosed pregnant and those apparently pregnant but undiagnosed. At least 63 days had elapsed since breeding and without reoccurrence of oestrum.

** Cows with a history of repeated breeding.

*** This cow had been previously bred 3 times.

These data were compiled from two breeding rings and the University herd. At the moment it has not been possible to have pregnancy diag-

noses on all cows inseminated with yolk-buffered semen. Two problems have come to our attention in this connection. One is the fact that there has been a "repeater" list (cows that require more than one service to settle) in this group of breeding cows. In these experiments the repeaters averaged slightly more than 40 per cent of the animals under observation. Secondly, a certain group of cows would apparently settle after service and skip several heat periods following insemination but later they show estrum and rebreed. Whether or not pregnancy occurred in these cows could not be determined. As far as we can ascertain these two problems are present in the herd irrespective of natural or artificial service used in the insemination. The yolk-buffer did not produce or prevent this condition. In view of the excellent results obtained in these experiments, it appears that the egg yolk constituents were completely metabolized or eliminated without harm to the cow.

One more point should be made clear. There is a great variation in the quality of bull semen. The yolk-buffer solution does not improve a poor sample of semen. We have noticed a distinct difference in the length of time semen can be preserved from bulls of different potency ratings. The bull that produces an excellent semen with high initial activity can be successfully stored for longer periods than the semen from a bull that produces a less vigorous semen sample.

## SUMMARY AND CONCLUSIONS

A method for the preservation of bull semen has been described. This method makes use of egg-yolk buffered at pH 6.75 as the pabulum for spermatozoa. By this means the fertilizing capacity of vigorous spermatozoa has been regularly maintained for periods in excess of 100 hours, if stored at 10° C. This method has been successfully used under practical conditions.

Under these environmental conditions bull semen can be successfully stored to give consistent preservation of its fertilizing capacity under practical conditions. Yolk-buffer maintains potency equivalent to the original semen for a variable period depending upon the quality of the fresh semen sample. Cows have been successfully bred with yolk-buffered semen stored for 150 to 180 hours.

## REFERENCES

(1) ANDERSON, J. Artificial insemination of sheep. I. J. Agr. Soc., 27: 143–150. 1937.

(2) BAKER, JOHN R. A fluid for mammalian sperm suspension. Quart. J. Exp. Physiol., 20: 67. 1930.

(3) BERNSHSTEIN, A., AND SHERGIN, N. P. Decomposition of hexose-phosphoric acid in semen. Orenburg Vet. Inst. U.S.S.R., 36. 1933.

(4) HATZIOLOS, B. The preservation of bull sperm for artificial insemination. Tierzücht. u. Züchtenngsbiol., 38: 199–354. 1937.

(5) HYME, J. Experiments on the viability of spermatozoa. Ceska Dermatol., 12: 350–353. 1932.

(6) IVANOV, E. E. Respiratory quotient of spermatozoa. Bull. Soc. Chem. Biol., 18: 1613. 1936; The energy of movement of spermatozoa. Ztschr. f. Züchtung., 20: 404. 1931; Effect of monohaloacetic acid on glucolysis and motility of spermatozoa. Biochem. Ztschr., 278: 101–104. 1935.

(7) MILOVANOV, V. K. The principles of artificial insemination. Leningrad. 1934.

(8) PHILLIPS, PAUL H. Preservation of bull semen. J. Biol. Chem., 130: 415. 1939.

(9) SHERGIN, N. P. The acidity of sperm. Problems of animal husbandry, U.S.S.R. No. 12: 121–122. 1935.

(10) WALTON, A. Preservation of fertilizing capacity of horse semen. Am. Soc. Animal Prod., 238. 1939.

(11) WINTERS, L. M., COMSTOCK, R. E., COLE, C. L., GREEN, W. W., AND BULIK, J. J. Artificial insemination of farm animals. Minn. Exp. Sta. Bull. No. 336. 1938.

# THE COAGULATION TEMPERATURE OF MILK AS AFFECTED BY pH, SALTS, EVAPORATION AND PREVIOUS HEAT TREATMENT

P. G. MILLER AND H. H. SOMMER
*Department of Dairy Industry, University of Wisconsin*

The stability of milk under various conditions has been studied from both a theoretical and practical standpoint. The milk has been subjected to temperatures ranging from room temperature to 150° C., and to the influence of salts and acids that are foreign as well as those normal to milk. Although valuable information has been added to our knowledge of milk, yet many theoretical and practical questions have not been answered which are significantly related to the stability of milk during pasteurization, boiling, preparation of various food-stuffs involving milk, and the manufacture of condensed and evaporated milks. The purpose of the work presented herein is to show the relationship between the pH and the coagulation temperature of milk ranging from about room temperature to the coagulation temperature of stable milk, and to show the effect of added salts, evaporation and previous heat treatment in displacing the "coagulation curve."

## HISTORICAL

It has been known for a long time that fresh milk would coagulate at elevated temperatures. Hammarsten (10) in 1874 reported that the coagulation temperature varied from 130 to 150° C. Cazeneuve and Haddon (5) attributed the curdling of milk at 130° C. to the formation of acids, mainly formic, from lactose oxidation. Bardach (1) failed to confirm this conclusion. Milroy (20) showed that heating slightly increased the acidity of milk. More recently Whittier and Benton (43, 44, 45) concluded that the rate of acid production during heating at 90 to 120° C. was a direct function of the time and temperature of heating, and lactose concentration. Their results indicate that acid production may become an important factor with long holding periods at elevated temperatures. Holm, Deysher and Evans (11) concluded that the relationship between time and temperature of coagulation was of a logarithmic nature.

The early workers, such as Stokes (33), Thörner (37), Rideal (25), Richmond and Harrison (24) and Kastle and Roberts (14), showed that the acidity-curdling temperature relationship varied considerably between milks. Recently Benton (2) reported that wide variations existed even in the milk from different quarters of the same udder. The aims of the present paper are most closely indicated by the work of Tapernoux and Katrandjieff (35) in which the change in titratable acidity, pH and approximate curdling temperature was determined for a sample of milk held at 14° C.

Received for publication November 16, 1939.

The early workers observed that not only an increase in acidity but also the addition of salts would lower the coagulation temperature of milk. Ringer (26) in 1890 was one of the first to report such findings. Conradi (6) found that the addition of 0.2 to 0.6 per cent calcium chloride would coagulate milk at 45 to 65° C., while larger or smaller amounts would not coagulate the milk. Loevenhart (19) likewise found an optimum concentration of $CoCl_2$, $CaCl_2$, $MnCl_2$, and $NiCl_2$ for the coagulation of milk at 40 to 60° C.

Within recent years the problem of the heat stability of milk has received considerable attention due to the development of the evaporated milk industry. Sommer and Hart (30) in 1919 studied the coagulation of milk from individual cows at 136° C. and found that some samples coagulated in 90 seconds while others failed to coagulate in 20 minutes. No relationship was found between the coagulation and the titratable acidity or pH of the fresh milk samples. The differences in coagulation were found to be due mainly to the relative amounts of calcium and magnesium as compared to the citrates and phosphates present in the milks. An excess of either of these two groups caused decreased stability; for maximum stability they must be present in the proper ratio or balance. Rogers, Deysher and Evans (27) in 1921 found no correlation between the coagulation of the raw mixed herd milk and the same milk after evaporation. They were also unable to find by means of chemical analysis any relation between the acid-base ratio in the raw milk and the coagulation temperature of the evaporated product. However, Sommer and Hart (31) in 1922 showed conclusively that evaporated milks could be stabilized by small (within limits of analytical experimental error) additions of citrates or phosphates and the same milks destabilized by calcium.

Various investigators have confirmed the conclusion that salts are an important factor in the heat stability of milk and milk products; namely, Benton and Albery (3), Whitaker (42), Holm, Webb and Deysher (12), Webb and Holm (40), Welch and Doan (41), and Tracy and Ruehe (38). The results of Webb and Holm, and Holm, Webb and Deysher have been summarized (18) to show three types of milk. All skim milks of normal concentration are reported to be stabilized by phosphate and destabilized by calcium. When the skim milks were concentrated to half their original weights, they obtained type I, which was destabilized by calcium and first stabilized, then destabilized by larger additions of phosphate; type II, destabilized by both calcium and phosphate; and type III, destabilized by phosphate and first stabilized, then destabilized by additions of calcium. Welch and Doan (41) found that milk from cows with subclinical mastitis reacted like type III milk.

Sommer and Hart (32) found cases where the milk could be improved by small additions of acids. These results have been confirmed by sev-

eral other investigators. Benton and Albery (3) found that within the pH range of 6.58 to 6.65 the salt balance changes had a marked effect, while outside this range, changes in pH were the predominant factor. They concluded each sample of milk had its own optimum of pH and salt balance. A similar conclusion was reached by Webb (39) and Ramsdall, Johnson and Evans (23).

The probable role of rennet-producing organisms in the heat coagulation of milk was studied by Frazier (9). The effect of renin, acid and calcium appeared to be additive.

Previous heat treatment has been shown to be an important factor in the heat stability of milk. Conradi (6) in 1901 found that if the milks were first heated to above 60° C., the coagulation on addition of calcium chloride occurred at temperatures of 8 to 12° C. lower than in the case of unheated controls. Webb and Holm (40) found that milks with a solids-not-fat content of around 13 per cent were not affected by forewarming, but milks of lower solids were destabilized while milks of higher concentrations were stabilized. Various other workers have reported beneficial effects upon the concentrated product from forewarming (27, 7, 17, 16, 32). The results show the optimum forewarming temperature to be near 100° C. when heated for ten minutes. The relationship between temperature, time, method of forewarming and concentration has not been satisfactorily determined.

The effects of forewarming may be attributed to a number of factors. Söldner (28) in 1888 showed that boiling caused a precipitation of a portion of the calcium phosphate from milk. This has been confirmed by many other investigators. Palmer (22) concluded it was the colloidal $CaHPO_4$ that was affected by heating, however, beneficial effects of forewarming suggest the precipitation of soluble calcium salts. Sommer and Hart (32) found larger amounts of soluble calcium in samples of milk heated at 180° F. than when heated at 210° F. Sommer (29) showed that part of the beneficial effect was due to the precipitation of albumin. Svedberg, Carpenter and Carpenter (34) found that the molecular weight of pure casein was doubled by heating to 40° C. On the other hand, Nichols, *et al.* (21) concluded that the particle-size distribution of the calcium caseinate in skim milk was not affected by preheating at 65 to 95° C. Howat and Wright (13) found that heating calcium caseinate at 120° C. for 1 to 5 hours liberated free phosphorus and nitrogen, lowered the base-binding capacity and liberated calcium with the result that the product was less heat stable.

## EXPERIMENTAL

One of the difficulties in studying the heat stability of milk is the lack of a satisfactory method for measuring the coagulation point of the milk. The results shown in figure 1 were obtained by forcing the milk by means

of air pressure through small glass tubing extended through a water bath in which the temperature was gradually increased, and allowing the heated milk to drip at a constant rate onto a black plate glass. The occurrence of coagulation was easily detected by observing the milk as it flowed over the black glass, and at the instant of coagulation the temperature of the water bath was taken as the coagulation temperature of the milk. An adaptation of this plan for measuring coagulation at elevated temperatures involved many difficulties and was abandoned.

The method finally deemed most satisfactory for high temperatures was a modification of the one used by Sommer and Hart (30). This consisted of sealing the milk in glass tubes made from 8 mm. pyrex tubing, placing the tubes of milk in a rack which was then immersed in a thermostatically controlled oil bath. The rack was clipped onto a shaft which was rocked back and forth by mechanical means so that the milk flowed from one end of the tubes to the other, with a pause at each position to allow complete flow of milk. Therefore, uniform agitation was obtained and at the same time the coagulation time could be detected by noting the flow of the milk. In order to decrease as much as possible the factor of acid production from lactose oxidation, a time interval just ample to allow the sample to reach the oil bath temperature was chosen. Thermocouple measurements showed that the milk came up to the temperature of the bath in 85 to 110 seconds; hence, the coagulation point or temperature was fixed as that temperature at which the milk would coagulate in just

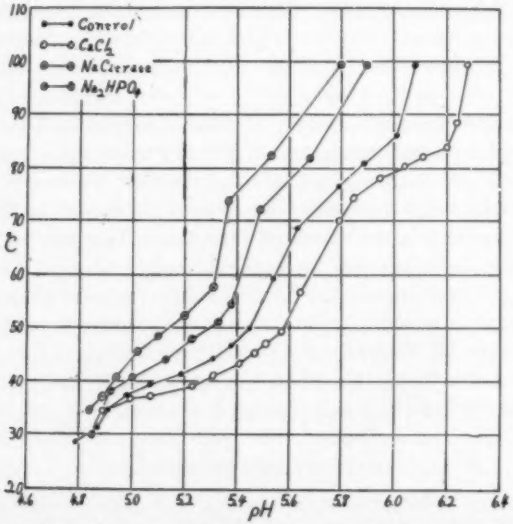



FIG. 1. The effect of salts and pH on the coagulation temperature of milk at temperatures below 100° C. 2 ml. M/4 salt added per 100 ml. skim milk.

two minutes from the time the sample was placed in the oil bath. Since numerous tests would be necessary to determine the temperature which would cause coagulation in exactly two minutes, the coagulation time was determined at temperatures above and below this time limit. These results were plotted, and the coagulation temperature for a two minute heating period was obtained by interpolation. For each sample a series of sub-samples was prepared ranging from about pH 5.0 to 6.8. The temperature required to produce coagulation in two minutes was determined for each sub-sample and plotted against the pH. The curve plotted in this manner is designated as the coagulation curve of the sample.

The acidification of sub-samples was accomplished by fermentation in the case of figure 1, and by acid additions in all subsequent experiments. This was done by adding diluted lactic acid in a very fine stream with the milk in vigorous agitation. To study the effect of the dilution involved, acidification of some samples was accomplished by agitating the milk in contact with air containing controlled amounts of HCl gas, with and without dilution.

Due to the amount of work involved, it was necessary to make up the samples one day, store them in the ice box and run the coagulation points and pH the following day. The pH was determined with the quinhydrone electrode, the temperature being maintained at 25° C. by means of a thermostatically controlled water bath.

A comparison of the methods of acidification (Curves G, H, I, Fig. 2) showed no detectable effect on the coagulation curve below pH 6.4. The control sample in figure 1, acidified by fermentation, shows a slightly different curve. Without further study it is not known to what extent the difference is due to other effects of fermentation, *e.g.*, citrate destruction, or to the method of heating.

Added salts displaced the coagulation curves as shown in figures 2 and 3. This displacement was into the more stable region by $Na_2HPO_4$, $Na_3C_6H_7O_5$ and $K_2CO_3$ and into the less stable region by $CaCl_2$ and $Ca(C_2H_3O_2)_2$, except at a pH above 6.5. The addition of phosphate, citrate or carbonate to the fresh skim milk (pH above 6.5) lowered the stability of the skim milks. The amount of calcium added also decreased the heat stability, but a smaller addition of calcium would have increased the heat stability of these skim milks as is revealed by results recorded in table 1. Since these milks at their initial pH were deficient in calcium for maximum heat stability, the first increases in acidity had an effect analogous to that of adding small amounts of calcium salts.

The results presented in figure 4 show that the addition of salts at a rate which produces maximum effects in the fresh milk, as indicated in table 1, has very little effect at lower pH. A portion of this sample condensed 2:1 illustrates the form of coagulation curve given by condensed

milk. The condensed portion reacted still less, but similar to that of the original, to the addition of phosphate, calcium and acid.

Figure 5 shows the effect of larger additions of salts on the coagulation curve of the skim milk sample before and after condensing. Adding various amounts of calcium and phosphate, from minute to large quantities, showed that the condensed portion was destabilized by both salts.

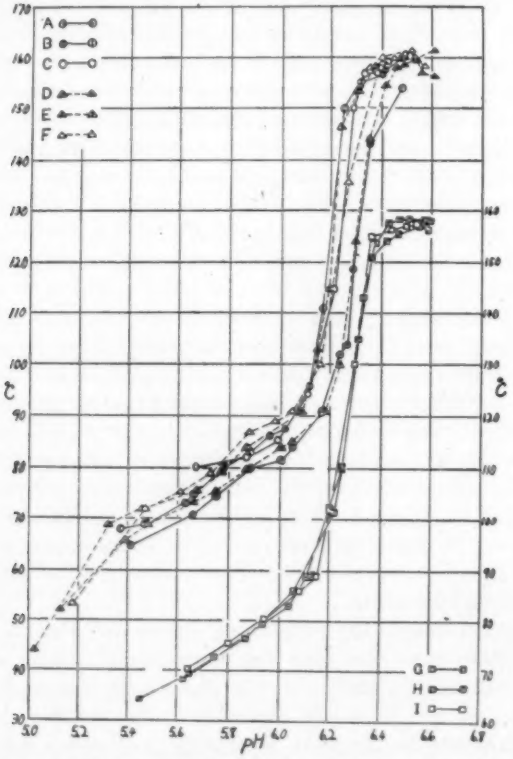



FIG. 2. The coagulation temperature of skim milk as affected by pH, salts and method of acidification.

Added to 100 ml. of skim milk (before acidified with HCl vapors) – 2 ml. of:

| | |
|---|---|
| A—water | D—water |
| B—M/4 $Na_2HPO_4$ | E—M/4 $Na_2PO_4$ |
| C—M/4 $K_2CO_3$ | F—M/4 $K_2CO_3$ |

Added to 100 ml. of skim milk:

G—5 ml. water; then acidified with HCl vapors.

H—5 ml. dilution; acidified with lactic acid solution.

I—no dilution; acidified with HCl vapors.

Temperature scale on the right relates to G, H, & I.

Temperature scale on the left relates to A, B, C, D, E, & F.

On the other hand, the curves show that the sample was increased in heat stability by adding acid.

The similarity of the effects of preheating and pH, and of evaporation and pH on the coagulation temperature of skim milk is illustrated in figure 6. Curve A gives the coagulation curve of the original skim milk and curve F shows how it was displaced merely by preheating. These are

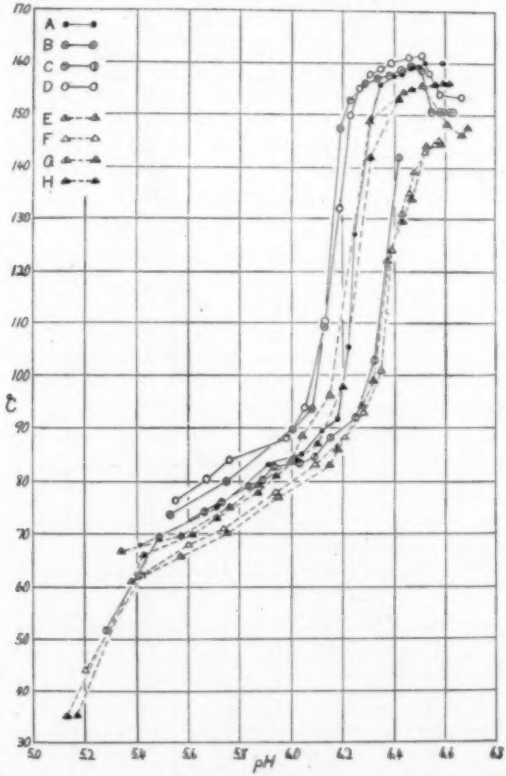



FIG. 3. The coagulation temperature of skim milk as affected by pH and salts.
Added to 100 ml. of skim milk (before acidified with HCl vapors) – 2 ml. of:

A—water
B—M/4 $Na_2HPO_4$
C—M/4 $CaCl_2$
D—M/4 $Na_3C_6H_5O_7$

Added to 100 ml. skim milk (before acidified with 3 ml. lactic acid solution) – 2 ml. of:

E—M/4 $CaCl_2$
F—M/4 $Ca(C_2H_3O_2)_2$ } pH above 6.4 by adding NaOH
G—M/4 $Na_3PO_4$
H—water

compared to the displacement by condensing the preheated sample (curve M) and by condensing the unpreheated sample (curve P). Heating at 90–98° C. for 30 minutes made the skim milk of normal solids content less stable to heat at pH values above 6.5 and below 6.1. The concentrated portions showed the same trend in that the preheated sample was less stable than the sample not preheated at pH values above 6.15 and below 5.75.

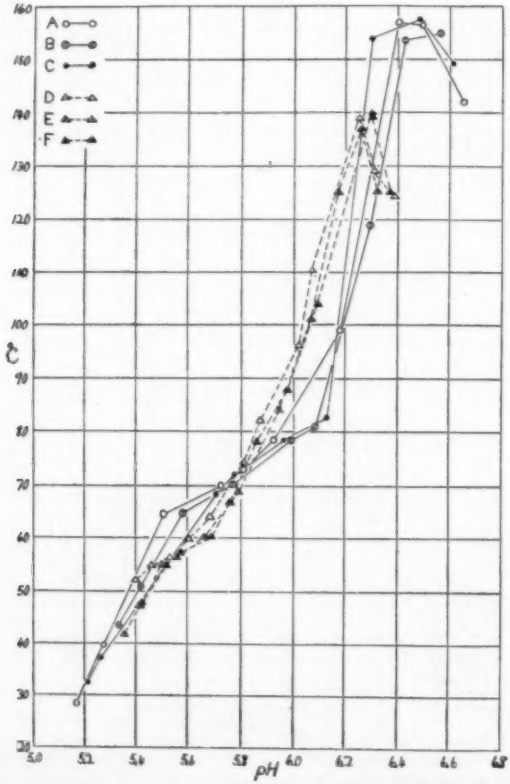



FIG. 4. The coagulation temperature of skim milk as affected by pH, salts, and evaporation.

Skim milk—to 100 ml. added (before acidifying with 4 ml. lactic acid) 0.8 ml. of:

A—M/4 $Na_2HPO_4$
B—M/4 $Ca(C_2H_3O_2)_2$
C—water

Skim milk preheated at 98° C. for 5 minutes and evaporated 2:1 at 59° C., 100 ml. portions treated as above:

D—M/4 $Na_2HPO_4$
E—M/4 $Ca(C_2H_3O_2)_2$
F—water

TABLE 1

*The effect of calcium and phosphate on the coagulation time of skim milk*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 25 ml. milk plus | ml. water | 0.0 | 0.6 | 0.55 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.0 |
| | ml. M/4 salt | 0.0 | 0.0 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 |
| | | Coagulation at 136° C. in minutes | | | | | | | | |
| Sample A | | | | | | | | | | |
| Calcium acetate | | 2: 40 | 3: 05 | 4: 25 | 21 | 47 | 45 | 45 | 2: 00 | 0: 35 |
| Disodium phosphate | | 2: 40 | 3: 05 | ..... | 2: 15 | ..... | 1: 50 | 1: 55 | 1: 55 | 2: 00 |
| Sample B | | | | | | | | | | |
| Calcium acetate | | 3: 05 | 3: 05 | 4: 20 | 13 | 34 | 29 | 28 | 27 | 0: 45 |
| Disodium phosphate | | 3: 05 | 3: 05 | ..... | 2: 30 | 2: 25 | 2: 20 | 2: 15 | 2: 20 | 2: 30 |

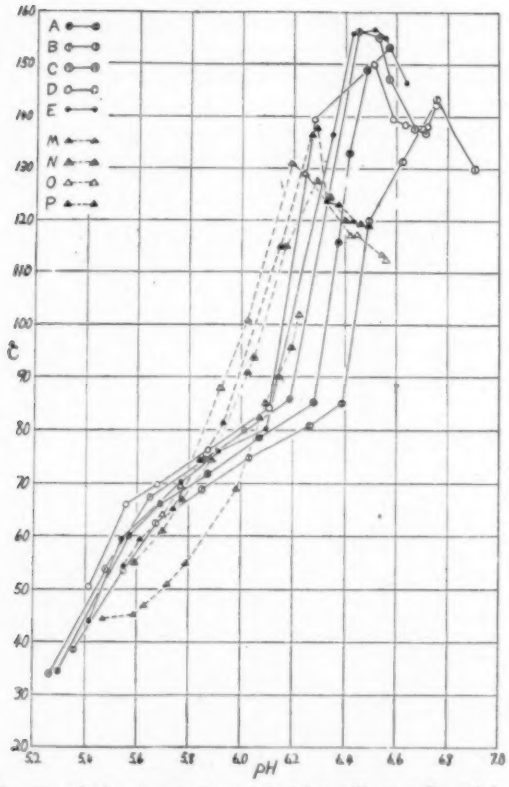



FIG. 5. The coagulation temperature of skim milk as affected by pH, salts and evaporation.

Skim milk—8.6% T.S.—to 100 ml. added (before acidifying with 2.8 ml. lactic acid solution) 1.2 ml. of:

A—M/4 $Ca(C_2H_3O_2)_2$
B—M/2 " —pH above 6.5 by adding NaOH
C—M/4 $Na_2HPO_4$
D—M/2 "
E—water

Skim milk—16.5% T.S.—100 ml. portions treated as above:

M—M/1 $Ca(C_2H_3O_2)_2$
N—M/4 $Na_2HPO_4$
O—M/1 "
P—water

The curves of figure 7 show that calcium and phosphate displaced the coagulation curve of the samples represented in figure 6 in the usual way; that is, after the milks have reached their maximum stability further increase in the H-ion concentration caused the phosphate to displace the

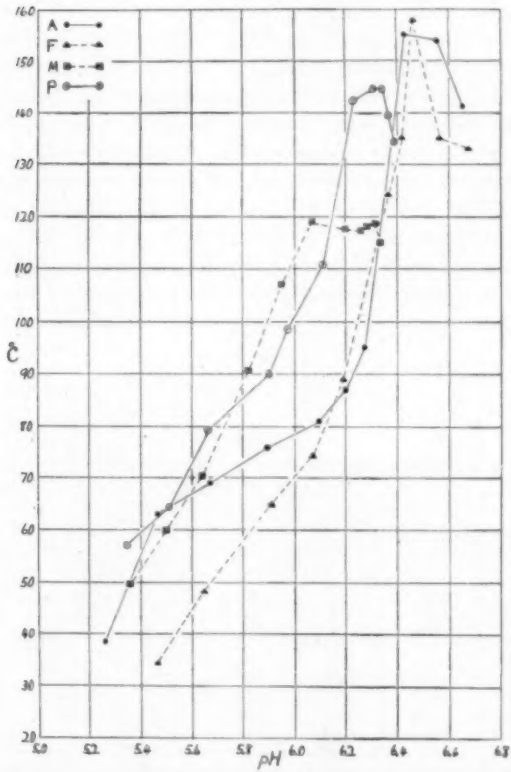



FIG. 6. The coagulation temperature of skim milk as affected by pH, preheating and evaporation.

A—Skim milk 8.7% T.S.; not preheated; diluted 4.4 ml. per 100 ml.

F—Skim milk 8.7% T.S.; preheated at 90–98° C. for 30 minutes; diluted 4.4 ml. per 100 ml.

M—Skim milk 20.1% T.S.; preheated at 90–98° C. for 30 minutes before evaporation; diluted 8 ml. per 100 ml.

P—Skim milk 19.6% T.S.; forewarmed only to 60° C. before evaporation; diluted 8 ml. per 100 ml.

the coagulation curve into the more stable region while calcium displaced the curve into the less stable region. A possible explanation of this is indicated by the results in table 2. It is shown that as the temperature of the milk is increased from 20 to 60° C., the pH of the milk is lowered. The presence of added calcium in the milk caused the pH of the milk to be decreased more rapidly while added phosphate caused the decrease to be less rapid than in the normal milk containing no added salts.

An increase in the acidity of milk with increased temperature was pointed out by Duncombe (8) in 1924. Zoller (46) had shown in 1920 that solutions of milk salts attained a higher pH after they had been heated in the presence of additional citrates. The changes in pH of various buffer solutions with temperature have been studied by many workers. As an example of the results, Kolthoff and Tekelenburg (15) reported that a 0.05 M potassium acid phthalate solution increased in pH from 3.94 at 18° C. to 4.05 at 60° C. and a 0.1 M tri-sodium citrate solution decreased in pH from 9.18 at 18° C. to 8.82 at 60° C. The significance to the heat stability of milk of various physico-chemical equilibria at elevated temperatures is further emphasized by the following formula for the iso-electric point of a simple monobasic, monoacidic ampholyte (36) :–

$$(H^+) = \sqrt{\frac{k_a k_w}{k_b}}$$

TABLE 2

*The effect of calcium and phosphate on the change in pH of milk with increased temperatures*

| Temperature | M/20 potassium acid phthalate | | 100 ml. skim milk plus 2 ml. of | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | water | | M/4 CaAc | | M/4 $Na_2HPO_4$ | |
| | pH | ±pH at 20° | pH | ±pH at 20° | pH | ±pH at 20° | pH | ±pH at 20° |
| 20 | 3.93 | ..... | 6.64 | ..... | 6.51 | ..... | 6.75 | ..... |
| 30 | 3.97 | 0.04 | 6.55 | 0.09 | 6.37 | 0.14 | 6.65 | 0.10 |
| 40 | 3.99 | 0.06 | 6.45 | 0.19 | 6.27 | 0.24 | 6.60 | 0.15 |
| 50 | 4.02 | 0.09 | 6.34 | 0.30 | 6.14 | 0.37 | 6.51 | 0.24 |
| 60 | 4.07 | 0.14 | 6.23 | 0.41 | 6.05 | 0.46 | 6.35 | 0.40 |

Note. Ecalomel N/10 from International Critical Tables *1*: 81. 1926. $E_h = 0.7175 - 0.00074t$ (4).

The value of $k_w$, the dissociation constant for water, increases markedly with temperature. The effect of temperature on $k_a$ and $k_b$ for milk proteins is not known. It is conceivable that coagulation at elevated temperatures may be due to shifts in these constants such that the iso-electric point is approached.

The effect of pH on the coagulation temperature of the various original skim milks represented in the previous curves is summarized in figure 8. Three of the skim milks were initially unstable. Merely by decreasing the pH, they were stabilized to a coagulation temperature above 152° C. Between pH 6.6 and 6.4 the coagulation temperature showed very little change with pH. Decreasing the pH from 6.4 to 6.2 rapidly decreased the coagulation temperature from near 155° C. to about 90° C. Then decreasing the pH from 6.2 to about 5.4 gave a very gradual decrease in coagulation temperature from about 90° C. to near 65° C. Points below pH 5.4 were

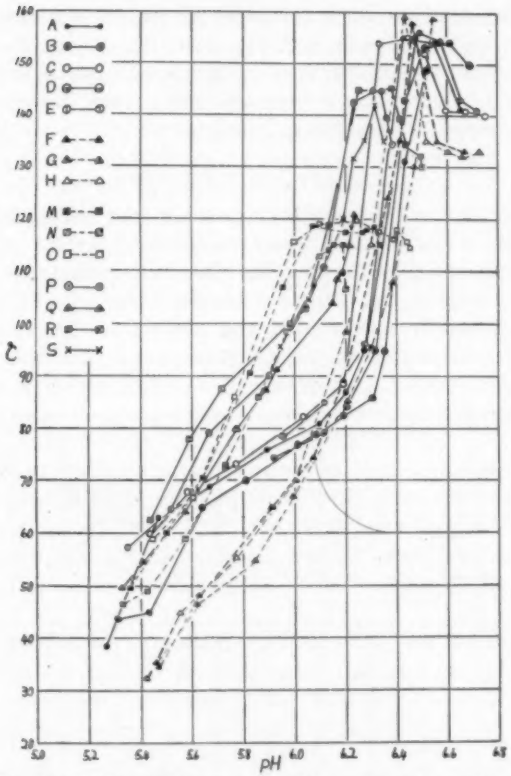



FIG. 7. The coagulation temperature of skim milk and of the evaporated skim milk as affected by pH, salts and heat treatment.

Skim milk—8.7% T.S.; not heated; to 100 ml. added (total dilution 4.4 ml. due to salt sol'n, lactic acid and water):

A—water
B—2 ml. M/4 calcium acetate
C—2 ml. M/4 di-sodium phosphate
D—0.8 ml. M/4 calcium acetate
E—0.8 ml. M/4 di-sodium phosphate

Skim milk—8.7% T.S.; heated for 30 min. at 90–98° C.; to 100 ml. added (total dilution 4.4 ml. etc.):

F—water
G—1.6 ml. M/4 calcium acetate
H—1.6 ml. M/4 di-sodium phosphate

Skim milk—20.1% T.S.; preheated 30 min. at 90–98° C.; to 100 ml. added (total dilution 8 ml. etc.):

M—water
N—4 ml. M/4 calcium acetate
O—4 ml. M/4 di-sodium phosphate

Skim milk—19.6% T.S.; forewarmed to 60° C.; to 100 ml. added (total dilution 8 ml. etc.):

P—water
Q—4 ml. M/4 calcium acetate
R—4 ml. M/4 di-sodium phosphate
S—1.2 ml. M/4 calcium acetate

not very definitely determined, however, the results indicate that decreasing the pH from 5.4 to about 5.2, decreased the coagulation temperature from about 65° C. to near 35° C. It also appears that decreasing the pH from 5.2 to 4.7, the iso-electric point of casein, would give another region of gradual decrease in coagulation temperature.

## SUMMARY AND CONCLUSIONS

1. Sealing the milk in small glass tubes and subjecting them to a rocking motion in an oil bath was found to be a convenient method for studying the heat coagulation of milk. The milk came up to the temperature of the oil bath in less than two minutes.

2. The relationship between pH and coagulation temperature of skim milk formed a characteristic coagulation curve. The milks were very sensitive to pH changes within two pH ranges; from approximately pH

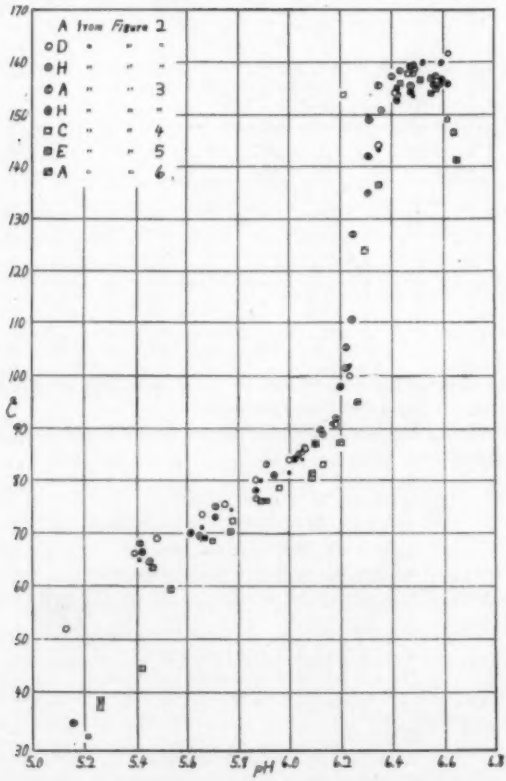



FIG. 8. The effect of pH on the coagulation temperature of skim milks represented in figures 2 to 7.

6.4 to 6.2 and to a lesser extent between pH 5.4 and 5.2. The maximum coagulation temperature of all the milks studied was above 152° C. The coagulation temperature of milks initially less stable than this was increased in heat stability by adding acid.

3. The presence of added salts displaced the coagulation curve; at pH values below 6.4, phosphate displaced the curve into the more stable region whereas calcium had the opposite effect. The effect at pH values above 6.4 depended upon the milk in question. The milks were initially destabilized by adding phosphate and the subsequent addition of acid restabilized the samples. The addition of small amounts of calcium stabilized the milks but larger additions destabilized the milks.

4. Concentrating and preheating displaced the coagulation curve of the milks in the same general manner, but the effect of concentrating was much greater than the effect of heat treatment alone. The presence of added salts displaced the coagulation curve for concentrated and preheated milks in a manner similar to that of the original milk.

5. The pH of the milk was decreased at increased temperatures; the presence of added calcium increased the effect of temperature changes while phosphate had the opposite effect. A more detailed study of various physico-chemical equilibria in milk at elevated temperatures would therefore appear to be a definite contribution to the problem of the heat stability of milk.

6. These conclusions are based on tests involving definite conditions, such as heating and holding so that coagulation occurred in two minutes, and do not necessarily represent the behavior of milk under other conditions.

## REFERENCES

(1) BARDACH, B. Über die Gerinnungsursache erhitzter Milch. Monatschr., 18: 199–216. 1897.

(2) BENTON, A. G. Variations in the milk from different quarters of the same udder: their significance in studies of coagulability. J. DAIRY SC., 12: 481–485. 1929.

(3) BENTON, A. G., AND ALBERY, H. G. Studies on the stability of evaporated milk during sterilization, with reference to the hydrogen ion concentration, alcohol test, and the addition of specific buffers. J. Biol. Chem., 68: 251–263. 1926.

(4) BIILMANN, E., AND KRARUP, I. The temperature coefficient of the quinhydrone electrode. J. Chem. Soc., 125: 1954–1956. 1924.

(5) CAZENEUVE, P., AND HADDON. Sur les causes de la coloration et de la coagulation du lait par la chaleur. Comptes rendus, 120: 1272–1273. 1895.

(6) CONRADI, H. Über den Einfluss erhöhter Temperaturen auf das Casein der Milch. Munch. Med. Wschr., 1: 175–177. 1901.

(7) DEYSHER, E. F., WEBB, B. H., AND HOLM, G. E. The relation of temperature and time of forewarming of milk to the heat stability of its evaporated product. J. DAIRY SC., 12: 80–89. 1929.

(8) DUNCOMBE, E. The influence of certain factors on the hydrogen ion concentration of milk. II. Temperature changes. J. DAIRY SC., 7: 245–248. 1924.

(9) FRAZIER, W. C. The influence of some bacterial enzymes on the heat coagulation of milk. J. DAIRY SC., 8: 370–389. 1925.

(10) HAMMARSTEN, O. Über den chemischen Verlauf bei der Gerinnung des Caseins mit Lab. (Maly's) Jahres-Bericht über die Fortschnitte der Thier-Chemie, 4: 135–154. 1874.

(11) HOLM, G. E., DEYSHER, E. E., AND EVANS, F. R. The relation of concentration and time to the temperature of coagulation of evaporated skim and whole milk. J. DAIRY SC., 6: 556–568. 1923.

(12) HOLM, G. E. WEBB, B. H., AND DEYSHER, E. F. The heat coagulation of milk. I. Variations in the compositions, heat stability and other tests of milk from four cows during the course of a lactation period. J. DAIRY SC., 15: 331–343. 1932.

(13) HOWAT, G. R. AND WRIGHT, N. C. The heat coagulation of caseinogen. I. The role of phosphorous cleavage. Biochem. J., 28: 1336–1345. 1934.

(14) KASTLE, J. H., AND ROBERTS, N. The chemistry of milk. II. Changes in the composition of milk. (1) Changes in milk produced by the action of heat and acids. U. S. Hygienic Laboratory Bull., 56: 328–332. 1909.

(15) KOLTHOFF, I. M. AND TEKELENBURG, F. The change of the pH in buffer mixtures at varying temperatures. Rec. trav. chim., 46: 33–41. 1927.

(16) LEIGHTON, A., AND DEYSHER, E. F. Factors influencing the heat coagulation of milk and the thickening of condensed milk. Proc. World's Dairy Congr. 2: 1276–1284. 1923.

(17) LEIGHTON, A., AND MUDGE, C. S. On the endothermic reaction which accompanies the appearance of a visible curd in milks coagulated by heat: a contribution to the theory of the heat coagulation of milk. J. Biol. Chem., 56: 53–73. 1923.

(18) LEIGHTON, A., AND WEBB, B. H. Heat coagulation of milk. Fundamentals of Dairy Science, 2nd ed., p. 211. 1935.

(19) LOEVENHART, A. S. Über die Gerinnung der Milch. Ztschr. Physiol. Chem. 41: 177–205. 1904.

(20) MILROY, T. H. The reaction and calcium content of milk as factors in the coagulation process. Biochem. J., 9: 215–228. 1915.

(21) NICHOLS, J. B., BAILEY, E. D., HOLM, G. E., GREENBANK, G. R., AND DEYSHER, E. F. The effect of preheating on the dispersity of calcium caseinate in skim milk. J. Phy. Chem., 35: 1303–1307. 1931.

(22) PALMER, L. S. The effect of heat on the calcium salts and rennet coagulability of cows, milk. Proc. Soc. Exp. Biol. & Med., 19: 137–142. 1921.

(23) RAMSDALL, G. A., JOHNSON, W. T. JR., AND EVANS, F. R. A test for the detection of milk unstable to heat. J. DAIRY SC., 14: 93–106. 1931.

(24) RICHMOND, H. D., AND HARRISON, J. B. P. Notes on sour milk. II. The point at which milk may be considered sour, and the rate of souring in the presence or absence of preservatives. Analyst, 25: 120–124. 1900.

(25) RIDEAL, S. The use and abuse of preservatives. Lancet, I. (new series): 228–230. 1900.

(26) RINGER, S. Regarding the action of lime salts on caseine and on milk. J. Physiol., 11: 464–477. 1890.

(27) ROGERS, L. A., DEYSHER, E. F., AND EVANS, F. R. The relation of acidity to the coagulation temperature of evaporated milk. J. DAIRY SC., 4: 294–309. 1921.

(28) SÖLDNER, F. Die Salze der Milch und ihre Beziehungen zu dem Verhalten des Kaseins. Landw. Vers. Sta. 35: 351–436. 1888.

(29) SOMMER, H. H. The heat coagulation of milk. Proc. of the World's Dairy Congress, 2: 1241–1248. 1923.

(30) SOMMER, H. H. AND HART, E. B. The heat coagulation of milk. J. Biol. Chem., 40: 137–151. 1919.

(31) SOMMER, H. H. AND HART, E. B. The heat coagulation of milk. J. DAIRY SC., 5: 525–543. 1922.

(32) SOMMER, H. H., AND HART, E. B. Heat coagulation of evaporated milk. Wis. Agr. Exp. Sta. Res. Bull. 67. 1926.

(33) STOKES, A. W. The influence of milk preservatives. Analyst, 16: 122–126. 1891.

(34) SVEDBERG, T., CARPENTER, L. M., AND CARPENTER, D. C. The molecular weight of casein. II. J. Am. Chem. Soc., 52: 701–710. 1930.

(35) TAPERNOUX, A., AND KATRANDJIEFF, K. Coagulation du lait par la chaleur eu fonction de son acidite. Compt. rend. biol., 101: 828–829. 1929.

(36) THOMAS, A. W. Colloid chemistry. McGraw-Hill Book Company, Inc., New York. 1934.

(37) THÖRENER, W. Bie welchem Säuregehalte gerinnt die Kuhmilch beim Aufkochen. Chem. Ztg., 15: 1108. 1891.

(38) TRACY, P. H., AND RUEHE, H. A. Factors affecting the heat coagulation of homogenized coffee cream. Ill. Agr. Expt. Sta. Bull. 352. 1930.

(39) WEBB, B. H. Heat coagulation of evaporated milk as affected by mixing different grades of raw milk. J. DAIRY SC., 11: 471–478. 1928.

(40) WEBB, B. H., AND HOLM, G. E. The heat coagulation of milk. II. The influence of various added salts upon the heat stabilities of milks of different concentrations. J. DAIRY SC., 15: 345–366. 1932.

(41) WELCH, R. C., AND DOAN, F. J. The heat stability of evaporated milk made from hard-curd milk, soft-curd milk and milk from mastitis infected udders. J. DAIRY SC., 18: 287–294. 1935.

(42) WHITAKER, R. The feathering of evaporated milk in hot coffee. J. DAIRY SC., 14: 177–188. 1931.

(43) WHITTIER, E. O., AND BENTON, A. G. The effect of heating on the hydrogen-ion concentration and on the titratable acidity of milk. J. DAIRY SC., 9: 481–488. 1926.

(44) WHITTIER, E. O., AND BENTON, A. G. The formation of acid in milk by heating. J. DAIRY SC., 10: 126–138. 1927.

(45) WHITTIER, E. O., AND BENTON, A. G. The rate of acid production in heated milk. J. DAIRY SC., 10: 343. 1927.

(46) ZOLLER, H. F. The buoying up of the equilibrium of milk salts during heat treatment. Science, 52: 614. 1920.

# THE KEEPING QUALITY OF BUTTER

C. M. SORENSEN

*National Butter Co., Dubuque, Iowa*

A factor in butter merchandising which is coming in for increasingly greater emphasis is the keeping quality of the product. The ability of any given lot of butter to retain its desirable flavor and odor characteristics through regular trade channels and into the hands of the ultimate consumer is a vital consideration in the manufacture and sale of high quality butter. The present discussion deals with the problem of butter keeping quality from the standpoint of the butter manufacturer and the butter merchandiser. It involves keeping quality examinations on 22,060 samples of creamery butter, each sample representing a regular commercial churning. The writer personally visited all creameries concerned at frequent intervals. The object of this work has been to determine by practical methods, the causes and elimination of defective keeping quality under commercial conditions.

## HISTORICAL

An extensive review of the literature pertaining to butter keeping quality was presented by Herried and associates (1) and later by Jacobsen (7). In the current study it was desirable to confine observations to butter defects which developed in fresh butter at ordinary refrigeration temperatures (30° F.–45° F.) in short periods of time. For this reason only those publications directly concerning defects such as putrid or cheesy, and rancid flavor developments are considered.

There is a considerable lack of agreement among numerous investigators as to the precise causes of off flavor developments. Also there are some differences of opinion with regard to the most satisfactory tests for evaluating the keeping quality of butter in the laboratory.

Hunziker (4) describes limburger flavor as a flavor development in butter which is not common, but which occurs spasmodically and is not infrequently epidemic. This author also emphasizes the importance of water purity as an important consideration in producing butter which keeps properly.

Hood (3) reproduced surface taint in butter by artificial inoculation of butter with water bacteria and indicated water contamination as a major cause of this type of difficulty with butter keeping quality.

Herried (1) and associates found that raw cream in a majority of cases contained organisms capable of producing cheese-like flavors in unsalted butter. Pure cultures isolated from this source and mixed cultures

Received for publication November 20, 1939.

of bacteria from raw cream and from creamery water were inoculated into sterile cream and were found to produce cheesy flavors in the resulting butter. Creamery water was sometimes found to contain organisms capable of producing cheesy flavors in unsalted butter when inoculated into sterile cream.

In a study of seventeen samples of rancid butter, Hussong and Associates (6) found *Pseudomoras fragi* present in 16 of the samples. This organism was found in numerous other dairy products. It was found to be capable of rapid growth at 10° C. (50° F.) to 30° C. (86° F.) but was killed by incubation for several days at 37° C. The presence of this organism, found to be easily killed by heat, in pasteurized products was taken as an indication of contamination subsequent to heat treatment.

Jacobsen (8) made a study of bacterial content of butter before and after storage and observed the keeping quality of salted and unsalted butter before and after storage for 90 days at –25° C. (–13° F.). Decreases in numbers of bacteria during storage were observed in both unsalted and salted butter but decreases in counts were greater in the salted butter. No defective keeping quality was found in the salted butter after storage but some of the unsalted made from the same churnings developed flavor defects upon being submitted to a keeping quality test involving holding samples in sterile jars at room temperature for 8 days.

Nelson (10) observed the changes in numbers of bacteria in butter held 7 days at 21° C. by the microscopic method and by the plate method. This investigator found that large numbers of gram negative rods were commonly associated with poor keeping quality. No correlation between keeping quality and plate counts of butter was observed.

Olson and Hammer (12) studied the keeping quality of butter in relation to churn contamination and found rancidity to be the most common defect developing in butter as a result of contaminated churns. Samples studied were held at 32° F. and 45° F. The flavor deterioration was found to occur at 45° F. more rapidly than at 32° F.

Olson (11) made studies on the keeping quality and bacterial counts of butter made using artificially contaminated water. A Sietz filter was employed for removal of bacteria from water before washing butter. No appreciable difference in numbers of bacteria in butter from filtered or unfiltered water was noted in the butter as judged by the plate count but significant differences in keeping quality of the butter were noted in favor of the filtered water.

Using an especially adapted technique, Long and Hammer (9) investigated the effect of moisture dispersion in butter on the growth of bacteria in butter. They observed the keeping quality of butter made from cream inoculated with organisms isolated from defective butter. The degree of division of moisture droplets in butter (working) was found to markedly

influence the rate of deterioration. It was found that almost without exception, thoroughly worked butter had by far the best keeping quality, underworked butter deteriorated very rapidly while moderately worked butter showed intermediate keeping quality values. This work doubtless explains the lack of correlation in some earlier observations on butter keeping quality and causes of butter deterioration.

Hammer (2) has observed cases of roquefort cheese-like flavor development in butter which was commonly caused by mold growth even though molds were not evident until microscopic examinations were made. This author (2) also made many keeping quality observations on samples containing various butter spoilage organisms and indicated that unfavorable keeping quality at 40° F. to 45° F. was duplicated at 70° F.

It is generally agreed that defects which develop in butter held at ordinary refrigeration temperatures (30° F. to 50° F.) can be duplicated in shorter periods of time at higher temperatures. This fact affords a means of determining the relative keeping quality possessed by a lot of butter by simply holding a small sample for seven days to two weeks at a temperature between 60 and 70° F.

Nelson (10) employed a holding test for 7 days at 21° C. to evaluate the keeping quality of a number of churnings of commercial butter and exhibition butter. Jacobsen (7) describes a keeping quality test at room temperature for 7 to 10 days using sterile glass jars for holding sample as a valuable means of evaluating the keeping properties of unsalted and salted butter.

Parsons (13) employed a keeping quality test using a temperature of 60° F. for 14 days on regular parchment wrapped prints to examine commercial churnings over a period of five years and submitted thousands of churnings to this test.

## KEEPING QUALITY TESTS

The present study employed a keeping quality test wherein a one-fourth pound sample of each churning was wrapped in commercial parchment wraps and held seven days at 68 to 70° F. in a thermostatically controlled cabinet. All samples were examined for flavor and odor defects at the end of this period. Samples were reported as satisfactory, fair or unsatisfactory. When off flavors developed they were classified as slightly or definitely cheesy, putrid, rancid, etc., according to the type and extent of flavor development. A total of 22,060 churnings of both salted butter, containing 1.8 to 2.3 per cent salt, and unsalted butter from more than 100 plants located in 8 midwestern dairy states were represented by the samples thus examined. These churnings were made and examined during the period February 1st to December 31st, 1938. Seven per cent of the total churnings examined were 93 score unsalted, about half of which were

unripened unsalted, the remainder being ripened unsalted. As might be expected, keeping quality of ripened unsalted was found to be superior to the unripened unsalted. In general it was found that when cheesy or putrid type flavors were encountered in unripened unsalted the flavor appeared very soon after manufacture. In several instances butter made from first quality cream developed this flavor before it had left the creamery. Rancid flavor development in unsalted butter usually developed more slowly. As will be seen from table 1, putrid or cheesy flavors in unsalted were usually very definite when encountered on keeping quality tests. Rancid flavors, however, occurred in varying degrees of intensity and somewhat less rapidly though the defect is doubtless equally serious in unsalted butter and apparently occurs more commonly.

Approximately 70 per cent of the churnings examined were 92 and 93 score quality salted butter, there being slightly more 93 score than 92 score. Approximately 7 per cent of the total samples were unsalted butter, the remaining 23 per cent representing 91 scores and below.

All churnings were from weekly shipments of fresh butter and were graded by an official government grader upon arrival. In examination of keeping quality tests after incubation no effort was made to place a numerical score upon the samples. All samples which did not retain their sale value according to original grade were classified as definitely off and unsatisfactory. A surprisingly close correlation between keeping quality tests and subsequent difficulty with the churnings tested was noted.

Where a defect was only slightly indicated after completion of the holding test, subsequent difficulty with the butter was very rarely encountered.

From table 1 it is plainly evident that putrid-cheesy type flavor development was by far the most frequently encountered keeping quality difficulty in salted butter. Tests indicated that with 76.62 per cent of the samples which showed unfavorable keeping quality this type of flavor and odor development was the cause. In addition, when this development occurred almost two out of three cases developed the defect to a very marked degree. The defect was as pronounced in low scoring undergrade churnings as it was in top grades.

Some investigators have classed cheesy flavors according to types of cheese which they resemble. Molds have been found to produce roquefort-like flavors in butter. Bacteria have been found to cause limburger or putrid-type flavors as well as cheddar cheese flavors of varying intensity. The terms putrid or cheesy as used in the present study indicate all types of cheesy flavor development but by far the largest proportion of these flavors encountered were of the putrid or limburger type, which are commonly referred to by the trade as proteolytic or protein decomposition flavors. In general it has been found that organisms commonly encoun-

TABLE 1

*Distribution of samples which developed unfavorabe keeping quality according to flavor development and original score. Figures represent per cent of total spoilage for each grade of butter*

| | Putrid cheesy flavors and odors | | | Rancid flavors and odors | | | Moldy, stale, tallowy and misc. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Slight | Definite | Total | Slight | Definite | Total | Slight | Definite | Total |
| 93 score unsalted (large majority of unripened unsalted) | 5.69 | 31.04 | 36.73 | 23.41 | 28.48 | 51.89 | 5.06 | 6.32 | 11.38 |
| 93 score salted | 26.04 | 49.52 | 75.52 | 8.68 | 3.55 | 12.23 | 9.64 | 2.57 | 12.21 |
| 92 score salted | 20.92 | 50.58 | 71.50 | 6.97 | 7.57 | 14.54 | 11.63 | 2.33 | 13.96 |
| 91 score salted | 27.15 | 41.72 | 68.87 | 5.30 | 8.61 | 13.91 | 11.26 | 5.96 | 17.22 |
| 90 score salted | 39.80 | 35.92 | 75.72 | 2.92 | 11.65 | 14.75 | 4.85 | 4.86 | 9.71 |
| 89 score or less | 12.50 | 43.75 | 56.25 | 3.13 | 28.12 | 31.25 | 0.00 | 12.50 | 12.50 |
| Total all grades salt butter | 23.64 | 52.98 | 76.62 | 5.95 | 5.60 | 11.55 | 8.33 | 3.50 | 11.83 |

tered where unfavorable keeping quality is concerned are of a type easily killed by ordinary pasteurization treatment of cream. Species of *Achromobacter* (1) and *Pseudomonas* (5) organisms have been frequently found in samples of butter having poor keeping quality. No cases of butter spoilage traceable to heat resistant (spore forming) bacteria have been encountered in the present study.

## SOURCES OF BUTTER SPOILAGE ORGANISMS

The most common and also the most serious butter spoilage concerned in the current study were traceable to contamination from the creamery water supply. Practically without exception, water contamination was responsible for typically putrid or limburger type flavor and odor development in both salted and unsalted butter. In creameries where the finest quality of cream was used and excellent pasteurization practice and sanitary practice on equipment was employed, water was repeatedly found to be the sole source of keeping quality difficulty. In such cases the common procedure was to submit water samples for bacteriological examination and to simultaneously inspect water tank systems, water pumps and wells. As a precautionary measure, water tanks were cleaned and the well, pipe lines, and tanks given a chlorine treatment using chlorinated lime or calcium hypochlorite compounds. It was found that in some cases treatment of the water tanks eliminated the difficulty without further changes. In these cases accumulation of relatively small amounts of sediment of a flocculent nature in the bottom of the tanks which gave off small amounts of gas were indicated as causing the contamination of the butter. Here also it was found that the water delivered from the pump was entirely satisfactory but was contaminated in passing through the storage tanks.

Another group of cases of water contamination was found where breaks in the well casing or drainage entering from the top of the well was responsible. In these instances steps were taken to render the casing tight and to seal off the upper end in such a way that liquid from condensation from the pump or moisture from the floor could not gain entrance to the casing. In addition, in order to disinfect the well itself a quantity of chlorinated lime mixed with thirty parts water was added to the well through the top of the casing. This mixture was allowed to remain in the well for 8 to 10 hours, then 300 to 400 gallons of water were pumped from the well through all outlets and into the storage tank. The pump was then operated until no chlorine odor was perceptible, after which the water was directed to usual use. In cases where localized contamination was encountered, this treatment proved effective in eliminating keeping quality difficulty.

Other instances of water contamination involving continuous seepage entering the water underground were encountered which did not respond

to well treatment. In such cases continuous chlorination by means of mechanical hypochlorite feeders were found effective. Several cases of water difficulty were encountered where creameries located in small municipalities did not have their own wells and were dependent upon the municipal water supply, which was not chlorinated. In such cases water treatment was accomplished by addition of measured amounts of hypochlorite to definite volumes of water placed in a cream vat or galvanized iron tank. This method was effective in eliminating defective keeping quality due to putrid flavor development in all of four such cases encountered.

Samples of butter from two creameries indicated as having water contamination difficulty due to continuous seepage to the well were submitted to laboratory examination and were found to contain *Achromobacter putrefaciens*.[1] This type of spoilage and in general spoilage traceable to water contamination was quite characteristic of putrid or limburger cheese type flavor. Consequently as this study progressed, the writer came to look for water contamination possibilities when this type of flavor development occurred and to apply chlorine treatment where indications of water contamination were found. Very gratifying results in eliminating keeping quality difficulty were attained by this approach to the problem.

Whether the addition of small amounts of chlorine to water used for washing butter are in any way detrimental to the product has not, as far as the writer is aware, been established. It is a well known fact, however, that many large creameries and dairies located in large cities constantly use water from municipal sources which contains from 0.1 to 0.5 parts per million of available chlorine. In the present study concentrations of from 0.2 to 5 parts per million of available chlorine were employed, the amount of chlorine used being proportioned to the contact period and to the pollution hazard. It was established that the official score was not impaired by the use of chlorine in water nor was there an instance of off flavor development on keeping quality tests in the case of butter made using chlorinated water which would indicate chlorine as having an effect in butter deterioration. The highest concentration tried was approximately 10 parts per million and even in this instance there was no apparent effect on the flavor score or the keeping quality of the butter. Occasional cases of cheesy type flavor development were traced to churns and vats, but in these instances the flavor development was neither as definite nor as characteristic as was the case when water contamination was involved.

The rancid flavor developments encountered were largely of the typically butyric or hydrolytic type. In these cases the churns were found to be the most consistent sources of difficulty. In most cases where the churn was indicated it was found that there was also trouble due to stickiness. This was corrected by careful attention to the method of wash-

[1] Samples examined by Dr. B. W. Hammer and Associates at Iowa State College.

ing the churn and by careful attention to temperature of the water used in washing. A practice which gives excellent results is to rinse the churn with water at 100° F. to 120° F. after use to remove any small amounts of butter. The churn is then rapidly filled about 1/3 full of water at 185° F. and revolved in high gear for 20 to 30 minutes, drained quickly and allowed to dry as quickly as possible. It is highly important that the water be at least 160° F. when the churn is drained after washing. Churns washed daily by this method have been found to be kept in excellent condition and difficulty from sticking is practically absent. This treatment does not seem to injure the wood as churns handled in this manner have been known to give satisfactory service for many years. The character of water with respect to hardness in some cases causes accumulation of scale which must be removed occasionally to prevent sticking. Packing glands on churns were found to be kept free of excessive contamination by the above practice in churn sanitation.

A few instances of rancid flavor development were traced to breaks in vat linings, covers and in vat coils. Only in extreme cases were packings on vats used for "holder" type pasteurization found to be in questionable condition. This may be largely due to the fact that a temperature of 165° F. for 30 minutes was commonly used in pasteurization, thereby giving the packing glands a considerable heat treatment. Only when the coil packings were leaking badly or nearly worn out was difficulty from this source indicated.

Considerable attention has been given to so-called sanitary pipe lines and cream pumps as keeping quality hazards in recent years. Properly handled, this equipment should never give trouble or excessive contamination. Some methods of handling this equipment, however, are open to criticism. In the present study, when difficulty was traced to this source, it was found that the reason was not a lack of cleanliness of the equipment. The most desirable practice in this regard was to arrange the system so that only one vat could be connected to the pump at any one time. Thorough scrubbing of the pipes and pumps after using, assembling just prior to use, and sterilizing the assembled system with "live" steam before use was found to be the best method of treatment. Certain types of cream pumps, namely those positive pumps employing oil cups on the inner side of the packing gland, were found to require special attention in sterilizing. The oil cup was discarded and replaced with a metal plug or petcock which was opened when the system was steamed. This practice was found to maintain these pumps in good sanitary condition. When such pumps are used for cream there is no need of the oil cup as butterfat in the cream gives adequate lubrication.

The occurrence of moldy, stale, tallowy and miscellaneous flavors were quite uncommon and were usually found to occur to only a slight degree.

None of these flavors were found to occur consistently in the butter from any single creamery. In contrast, the flavors of the putrid, cheesy or rancid types were found to occur regularly in specific creameries and when eliminated did not reoccur.

For the purpose of illustration specific cases of keeping quality difficulty are subsequently outlined.

### *Creamery A*

A creamery producing more than 75 per cent of 93 score unripened salted butter was found to have occasional difficulty with putrid flavor development. A complete inspection on all equipment revealed satisfactory conditions and satisfactory processing practice. Cleaning and sterilizing the water tank and careful attention to workmanship were the recommendations made and the trouble disappeared. About one month later the plant began manufacturing unsalted butter and the first shipment revealed several churnings which developed putrid flavor and odor. The well was treated immediately with chlorine by adding 3 lbs. of chlorinated lime (24 per cent available chlorine) mixed in 20 gallons of water to the well casing.[2] This mixture was followed by the addition of about 100 gallons of water.

A small amount of water was then pumped through the piping system leaving all outlets open. The well was then allowed to remain undisturbed for 10 hours. After this, sufficient water was pumped to render the flow free of lime. About 400 gallons accomplished this, after which the water was pumped to the storage tank—the tanks having been cleaned and sterilized previously. Immediately following this treatment, keeping quality difficulty in the butter completely disappeared and in more than 10 months no further occurrence of defective keeping quality has appeared. Earlier efforts to eliminate the trouble by simply treating the tanks had proven unsuccessful.

In this case it is thought that accumulation of material above the water pipe level in the well casing was responsible for the trouble, the water level remaining quite a distance above the pipe intake even though the pump was in operation. Stopping and starting the pump, of course, caused a change in the height of water in the casing. The addition of the chlorine mixture is assumed to have affected sterilization of the casing itself and, in addition, to have carried down accumulated solids by mechanical action thus removing a hazard to butter keeping quality.

### *Creamery B*

This case involved a creamery which was entirely dependent upon a small municipal water supply which was not chemically treated, but which

[2] Recommended and successfully used in treatment of wells at creameries, food plants, and canneries by G. A. Vacha, Chief Bacteriologist of the Minn. Dept. of Agr., Dairy and Food.

was taken from a 200 ft. well. The creamery manufactures a high percentage of 93 score butter and regularly maintains a salt content of 2.0 per cent to 2.3 per cent by analysis. A sudden appearance of a large proportion of unfavorable keeping quality tests showing typical putrid flavor development occurred. The writer visited the plant immediately and instituted a practice of treating all water used in connection with the pasteurized cream and the butter with one part per million of available chlorine. This was done by measuring water into a clean vat and adding measured amounts of calcium hypochlorite powder. Water used in washing the butter and in standardizing the moisture content of the butter was taken from this vat. The above procedure has since been carefully followed out and no further occurrence of defective keeping quality has been encountered. At the time the treatment was begun water samples were submitted to the state bacteriological laboratory. These samples, representing the water as drawn from the city lines at the creamery, were reported as being very unsatisfactory for use in butter due to the presence of excessive numbers of bacteria and in particular large numbers of proteolytic bacteria as measured by plate counts on skimmilk agar.

*Creamery C*

This case involves a creamery where the water supply was found to be affected by continuous underground seepage apparently caused by proximity of swampy land and the use of a large cess-pool for sewage disposal some years previous. At any rate, a new and deeper well was installed at the first appearance of difficulty. This installation was effective only temporarily, the trouble again appearing two months later. Routine bacteriological examination of the water directly from the well failed to show appreciable numbers of bacteria. However, treatment of the water used for each churning was the only method whereby the keeping quality of the butter could be maintained. Samples of water drawn on seven consecutive days failed to yield significantly high bacteria or yeast and mold counts. On the basis of these findings a few churnings of butter were made without the use of treated water. Immediately the trouble with keeping quality reappeared. A mechanical hypochlorite solution feeder was then installed and adjusted to deliver water containing 3 p.p.m. of available chlorine. This apparatus has been maintained in continuous operation and no occurrence of unfavorable keeping quality has appeared since that time. A number of other instances of application of regular chlorination of water have proven equally beneficial. It may be added that bacteriological examination of water even when excessive bacteria counts were obtained yielded negative tests for colon-typhoid type organisms in all but one of the cases encountered.

WATER PURITY

When a new well is installed it is very commonly assumed that depth and absence of conspicuous pollution possibilities will render the water obtained satisfactory. As previously pointed out the presence of colon group organisms was detected in only one of the cases studied. On this basis then the water would be considered quite satisfactory for use from a public health standpoint. However, it is evident that spoilage can be carried by water even though public health authorities find it satisfactory. Unquestionably several factors contribute to the bacteriological condition of well water. During the period the current observations were made the mid-west dairy section received an abundant rainfall, which followed several years of relatively little rainfall. Fluctuations in the ground water level and the geologic makeup of subsurface strata are factors which doubtless effect the drainage of surface water to subterranean water levels. Periods of relative drought followed by periods of excessive rainfall are known to effect the pressure on ground water. Increases in pressure cause an increase in the amount of suspended matter water will carry. It is by no means beyond possibility that the amount of rainfall can be correlated to some degree with the presence of excessive numbers of micro-organisms in well water, particularly from comparatively shallow wells. Where the subsurface strata is predominantly sandy and the water table is relatively near the surface, there are good possibilities for contamination from surface drainage. In other areas where the substrata is predominantly limestone surface drainage is afforded relatively little filtration and thus may prove extremely important in water purity. Some suggestions are thus evident with respect to previously reported spasmodic and epidemic appearance of putrid or limburger type spoilage in butter.

There may be methods of water treatment which are more readily adaptable to use in small creameries than chlorination. However, the general use of chlorine and hypochlorite compounds in water treatment and its low cost appears to give the logical answer to water purity problems.

There are several methods which might be used in treating water against spoilage organisms. These include the ozone process, ultra violet treatment, the "electro katadyn" process involving ionic silver and filtration by means of a special filter for removing organisms. Very limited information is available on these methods and even less is known concerning their suitability for creamery use.

Pasteurization of water has previously been successfully used in creamery water treatment. This method, however, is somewhat cumbersome and quite expensive compared to chlorine treatment and if practiced regularly requires special equipment or considerable inconvenience. While there is the possibility that chlorine in water might have an effect on butter,

the use of small concentrations (0.2 to 5 p.p.m.) have not in the present study been found to effect the flavor or sale value of the product, but on the contrary have enhanced the value of butter supplies where unfavorable keeping quality was experienced prior to water treatment. In this connection Hunziker (5) has indicated that chlorine concentrations of 25 to 35 p.p.m. do not injure the quality of butter.

According to present information there is no reason to fear detrimental effects of small amounts of chlorine used in wash water upon the resulting butter. It may be added that the writer has repeatedly made colorimetric tests for available chlorine on wash water drained from the churn and was unable to detect any free chlorine even though the wash water before being placed in the churn contained 5 p.p.m. of residual available chlorine. It appears that the buttermilk diluted out by the wash water effects dissipation of the available chlorine immediately as the chlorinated water enters the churn.

## RANCIDITY IN BUTTER

Several cases of rancid flavor development in butter submitted to keeping quality tests were encountered. The common appearance of one, two or three samples showing this defect found in weekly shipments was the rule. In one case, however, a very serious outbreak of rancidity development where virtually every churning in an entire shipment was defective in keeping quality was encountered. This was subsequently traced to a sticky churn and a defective packing gland on the cream pump. Another instance of serious rancidity development was subsequently traced to vats in need of repair. In another case where only a very occasional case of rancidity occurred, it was found that each time the churning could be traced back to one particular vat. Inspection of this vat revealed a broken cover lining which was immediately repaired. This done, no further cases of this flavor development occurred. In a few instances cheesy type flavors were occasionally found where outbreaks of rancidity were encountered. These were usually far milder and less pronounced than was the case when cheesiness caused from water contamination occurred.

As a result of the foregoing experience, it may be indicated that where typically putrid or limburger type flavor development occurs in butter held on keeping quality tests, an impure water supply is commonly the ultimate source of the trouble. Where rancidity is encountered the most likely source is the sanitary condition of equipment, particularly the churn, with the vats, cream pumps and pipe lines next in importance in the order named.

Other types of flavor developments did not occur in any significant frequency but were limited largely to butter scoring 91 or less when fresh (see table 1). Two cases of mold development were traced to storage of butter in tubs in a wet cooler. In one of these, failure to sterilize parch-

ment liners was a major contributing cause. Stale flavors in many cases were found to a very slight degree in fresh butter and were slightly intensified in holding. Observation of the held samples at approximately room temperature may have been partially responsible for this condition. Occurrence of tallowy flavors was relatively rare and when found these flavors were present only to a slight degree.

For the most part butter represented by the samples examined was marketed as fresh butter and was consumed within six weeks from the date of churning. Butter which was stored was selected on the basis of creameries whose keeping quality records were satisfactory. After 3 to 4 months storage at –10° F. keeping quality tests were satisfactory on both salted and unripened unsalted butter.

While the keeping quality test employed herein may leave some points to be desired, there is no question as to its value in commercial practice. It is not practical in many instances to await results of the test before marketing any given lot of butter. Nevertheless an early knowledge of a tendency for butter from any source to develop unfavorable keeping quality is of very material value in preventing excessive losses as corrective measures can be brought to bear at the earliest moment. This test may well occupy a position in the butter industry similar to that held by the bacteria count of milk in that although the product may be disposed of before results of the examination are known, definite benefits of the tests on subsequent production are forthcoming before an appreciable amount of the product is placed in trade channels. The keeping quality test for butter can be conducted at the point of manufacture with no special equipment thus bringing practical bacteriological control methods to the creamery itself. Undoubtedly this test is one of the major contributions to the butter industry in recent years and bids fair to find increasingly greater application.

## SUMMARY

The results of keeping quality determinations by means of holding parchment-wrapped samples of commercial butter at 68° F. to 70° F. for seven days are presented and discussed. Twenty-two thousand sixty churnings of commercial butter were submitted to examination and personal visits were made to creameries whose butter showed unfavorable keeping quality. Putrid and cheesy type flavor development was found to be the most frequently encountered keeping quality difficulty. The major cause of this difficulty was traced to contamination of the creamery water supply with spoilage types of bacteria. Rancid flavor development was also found to be an important cause of defective keeping quality. This defect was most frequently traceable to equipment, notably churns, vats, sanitary pipe lines and cream pumps. Comparatively few cases of mold, staleness or tallowy flavor development were encountered.

Defective keeping quality was eliminated in cases of water contamination by chlorine treatment using hypochlorites. Repairs in equipment and changes in plant practice were found to eliminate rancid flavor development.

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. B. W. Hammer for his helpful suggestions and advice. To G. A. Vacha, Chief Bacteriologist of the Minnesota Department of Agriculture, Dairy and Food, whose valuable suggestions were freely offered, to Mr. H. R. Meier, Federal State Butter Grader, and Mr. M. C. Escher for their fine cooperation during the progress of this work, the writer is duly grateful.

REFERENCES

(1) Herried, E. O., Macy, H., and Combs, W. B. The microbiology of cheese-like flavors in unsalted butter. Minn. Agr. Exp. Sta. Tech. Bull. 97. 1934.
(2) Hammer, B. W. By correspondence. 1938.
(3) Hood, E. G. Surface taint. Address West. Can. Dairy Convention. 1927.
(4) Hunziker, O. F. The butter industry. Published by the author, La Grange, Illinois. 1927.
(5) Hunziker, O. F. Handling cream between pasteurizer and churn. The Creamery J., 50: 3, 5. Mar. 1939.
(6) Hussong, R. V., Long, H. F., and Hammer, B. W. Classification of the organisms important in dairy products. II. Pseudomonas fragi. Ia. Agr. Exp. Sta. Res. Bull. 225. 1937.
(7) Jacobsen, D. H. A holding test at room temperature as an indication of keeping quality of butter in storage. S. Dak. Agr. Exp. Sta. Bull. 308. 1937.
(8) Jacobsen, D. H. Bacterial content and keeping quality of butter after removal from storage. J. Dairy Sc., 21: 187–193. 1938.
(9) Long, H. F., and Hammer, B. W. The effect of moisture dispersion in butter on the growth of bacteria. Ia. Agr. Exp. Sta. Res. Bull. 246. 1938.
(10) Nelson, J. A. The correlation between the organisms found microscopically and the bacteriological deterioration of butter. Mont. Agr. Exp. Sta. Bull. 267. 1932.
(11) Olson, H. C. The influence of filtration of inoculated water on bacterial count and keeping quality of butter. B. W. Hammer. Panegyric. Collegiate Press, Inc., Ames, Ia. 1937.
(12) Olson, H. C., and Hammer, B. W. Bacteriology of butter. V. Studies on microorganisms in churns. Ia. Agr. Exp. Sta. Res. Bull. 159. 1933.
(13) Parsons, C. H. How to determine the keeping quality of butter. Nat. Butter and Cheese. J. 29: 7, 6 Apr. 1938.

# EXTREME RARITY OF CANCER IN THE COW'S UDDER: A NEGATIVE FINDING OF VITAL INTEREST TO THE DAIRY INDUSTRY AND TO THE CONSUMER

W. W. SWETT
*Senior Dairy Husbandman*

C. A. MATTHEWS
*Assistant Dairy Husbandman*

AND

R. R. GRAVES
*Chief, Division Dairy Cattle Breeding, Feeding and Management Investigations, Bureau of Dairy Industry, United States Department of Agriculture*

In connection with the investigations in the Bureau of Dairy Industry of the interrelation between conformation, anatomy and producing ability of cows, the udders of 313 cows of lactating age, and of 105 heifers and freemartins, have been filled with formalin, frozen, cut into sections, the sections photographed and otherwise studied in detail. This prescribed program brings to light any lesions, growths or other abnormalities that these udders may contain. Many of the lesions that have been found are thought to have resulted from mastitis but in no case has a cancerous growth been found. This paper describes unusual lesions that were found in two udders and discusses the highly important subject of the cow's apparent lack of susceptibility to mammary cancer.

Cow No. 656 made a 365-day production record at 2 years 4 months of 9,713 pounds of milk and 474 pounds of butterfat. There is no record of any udder trouble that required treatment during the first three lactation periods. During the fifth month of the fourth lactation it was noted on one occasion that the udder was tender and the right front quarter was enlarged, but this condition apparently disappeared in a short time.

The cow commenced her fifth lactation with a heavy milk flow. For the first 89 days the average daily milk production was 49 pounds. During the fourth month of lactation, on June 28, 1932, the right front quarter was reported as being swollen and hot. During the fifth month of lactation some indications of mastitis were noted in the left side of the udder, and at approximately 6 months after calving the right front quarter was hard and no milk was obtainable from it. This condition continued during the remainder of the lactation. After the disturbance in June the cow's total milk production was greatly reduced. Throughout the sixth lactation period the right front quarter was entirely inactive and her total production was low.

She commenced her seventh lactation period with a high level of production, averaging 48.6 pounds of milk daily during the first 92 days. Milk, which appeared to be normal, was obtained from the right front quarter that had been inactive through part of the fifth and all of the sixth lactation periods. This quarter was still very much enlarged. About 2 weeks after

Received for publication November 20, 1939.

calving a trace of flakes was noted by strip cup examination of milk from the right front quarter. The same condition was noted 4 weeks later. No other disturbance in the udder was recorded until she had been in milk for 105 days, when the previously unaffected right rear quarter became hard, the cow went off feed, and the udder dried up in all quarters. She was slaughtered on the 121st day of the lactation period. There appeared to be no swelling in the left half of the udder, which was rated very high in quality. However, the right front quarter was greatly enlarged and broken away from the abdominal wall and the right rear quarter was enlarged.

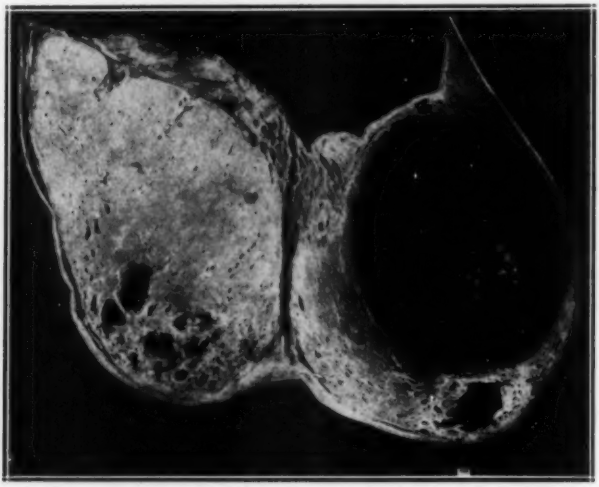


FIG. 1. A section through the front quarters of the udder of cow No. 656 showing an enormous abscess in the right front quarter which is entirely separated from the cistern and the adjacent gland tissue by a wall having approximately the same thickness as the hide covering the udder. The marked enlargement of the right front quarter distorted the udder so that the two front teats were not in the same transverse plane. This accounts for the absence of the left front teat on the section shown in figure 1.

The gross structure of the two front quarters is shown in figure 1. The left quarter does not appear to be abnormal. The right front quarter contained an enormous abscess which apparently had become entirely separated from the gland tissue by a wall having approximately the same thickness as the hide covering the udder. The abscess contained a large quantity of debris. Ventrally and medially to the abscess was an area of gland tissue that looked much the same as the tissue in corresponding areas in the adjacent left front quarter. Apparently this tissue was the source of the milk obtained from this quarter during the early months of the seventh lactation period. The fact that milk secretion was resumed in this quarter after

approximately eighteen months of inactivity during two consecutive lactation periods is very unusual.

The result of the mastitis infection which attacked the right rear quarter about 2 weeks before the cow went dry and was slaughtered, is indicated in figure 2. The gross structure of the tissue in the left rear quarter, like that in the left front quarter, appears to have been unaffected by the disturbance which caused complete cessation of lactation in this udder. It is possible, of course, that a histopathological study of the tissues in the left front and left rear quarters, would show that they had been affected by the infection.

FIG. 2. A section through the rear quarters of the udder of cow No. 656 showing the condition of the tissue in the right rear quarter after an acute attack of mastitis which occurred 3½ months after seventh calving.

Another unusual case is that of cow No. 1011. Some mastitis was noted 16 days after she calved the first time but this condition promptly disappeared. All four quarters were affected by mastitis at approximately 7 and

9 months after first calving but the disturbance did not appear to be marked or persistent. Her record for 305 days at 2 years 7 months of age amounted to 4,782 pounds of milk and 280 pounds of butterfat.

During her second lactation, she completed a 365-day record of 8,343 pounds of milk and 480 pounds of butterfat. Apparently the udder was in good condition except for traces of flakes, on strip-cup examination, in three of the quarters at different times during the last 3 months of this lactation period.

During the third lactation no disturbances were recorded aside from traces of flakes in the milk during the 6th and 7th months. She milked from all four quarters, but the left front and right rear quarters were relatively "light." Her production was comparatively low and she continued in lactation for only 255 days.

On the day of her fourth calving palpation by a pathologist indicated mastitis in all four quarters. Brom-thymol reactions on the same day were positive in all quarters. At weekly strip-cup examinations she showed marked mastitis in the right front quarter, the milk from that quarter becoming watery at 92 days. At 114 days the strip-cup examination indicated very marked mastitis in the left front quarter. The right front quarter was dry and the left front quarter was discharging pus 119 days after calving. She was milked for only 126 days.

About 2 months before her fifth calving the right rear quarter was hard and the tissue was harsh. A month later there was a definite lump in this quarter. The swollen condition continued until calving. Pus was discharged from this quarter before calving. Milk was not obtainable from any of the quarters after calving, even after probing by a veterinarian in an effort to remove or puncture the obstructions which were believed to have been formed in the region of the cisterns.

She was slaughtered July 7, 1936, seven days after calving. Examination of the udder before slaughter indicated that the cisterns in all except the right rear quarter, which was discharging pus, were completely walled off by what appeared to be a layer of fibrous tissue that destroyed all communication between the teat canals and the glandular tissue.

Immediately after the cow was slaughtered the udder was removed and prepared for post-mortem udder studies. The excised udder weighed 82.2 pounds. Because of the unsuccessful attempt to obtain milk by probing before the cow was slaughtered, a sheath knife (bistoury caché) was inserted into each teat in an effort to make a passage for the introduction of formalin. The toughness of the fiber is indicated by the fact that the bistoury would not go beyond the obstruction until the blade was released. The unsheathed blade, however, cut through a layer that was estimated to be about 1 inch in thickness, after which there was no appreciable resistance. The instrument was inserted approximately 4 inches beyond the point where

the teat was attached to the udder, and rotated with the blade extended. The same procedure was followed in each quarter. The filling of the udder was not entirely successful. It was thought at the time that a sufficient quantity of formalin had been retained to insure adequate preservation. When the frozen udder was sectioned, however, it was found that some of the areas most distant from the teats were not well preserved.

Examination of the sectioned udder showed that none of the four quarters had visible cisterns. The areas normally occupied by cisterns were filled with fibrous tissue (figs. 3 and 4). The marks made by the bistoury are

FIG. 3. Growth of fibrous tissue in the region of the cisterns of both rear quarters of cow No. 1011 had cut off all communication between the teat canal and the gland tissue. Note the channels leading upw· ·om the teats, which presumably were made by the bistoury. Note also the absenc .sterns and the extreme scarcity of ducts in both rear quarters.

visible in figure 3. A heavy-walled abscess was found near the base of the right rear quarter. Apparently this abscess had a small drainage outlet

FIG. 4. Shows an irregular-shaped transverse fissure in the left front quarter of the udder of cow No. 1011. Note also the fibrous tissue growth above the teat, the absence of cisterns and the scarcity of ducts in both front quarters. The dark areas near the top of sections shown in figures 3 and 4 are discolorations resulting from retained blood in the immediate vicinity of the large blood vessels.

into the teat which resulted in the discharge of pus before and after fifth calving.

A peculiar vertical, longitudinal split in the gland tissue was found in the left rear quarter. It commenced somewhat posterior to the rear teat and extended forward for a distance of some 7 or 8 inches, finally became diffused, and disappeared in the connective tissue adjacent to the median septum. The cleft reached a maximum depth of about 8 inches and a width of more than 2 inches. Another pocket or split was found in the left front quarter. In position it was vertical and transverse instead of longitudinal and was located slightly posterior to a vertical plane through the front teats. A general idea of its size, location and general appearance is given by figure 4. It was confined entirely within two of the cut sections, each of which was about 1 inch thick. Except for its color and position it appeared much like the cleft in the left rear quarter but they were several inches apart and there was no detectable connection between them. The cause of the clefts in the two left quarters is not known. Nothing of a similar nature has been found among the hundreds of udders studied. There is no reason to believe that the pressure used in injecting the formalin was responsible. On the other hand, the occurrence of severe mastitis had not been recorded in the left rear quarter, although severe mastitis and suppuration were in evidence in the left front quarter a few days before milking was terminated about 4 months after fourth calving.

Not only were the cisterns completely replaced by fibrous tissue but there was a very marked scarcity of ducts even in the areas near the cisterns where the ducts are generally abundant and often of good size. It was not determined definitely whether the udder was naturally dense throughout or whether the larger ducts, like the cisterns, had been replaced with other tissue. The fact that there was so little fibrous or scar tissue with the exception of the comparatively thin layer above each teat leads one to believe that numerous large ducts were never present in this udder.

Sometimes lesions and other abnormalities are found when udders are sectioned, that were not known or suspected to exist. A number of cases of small abscesses, cysts and deposits of various kinds have been found for which no plausible explanation can be found in the recorded history of the cow.

The two cases that have been discussed and illustrated show what are perhaps the most unusual abnormalities in udder structure that have been found in our work. The remainder of this paper is prompted by negative findings in these udder studies, that is, the absence of cancerous growths.

As stated at the beginning of this paper, 418 udders from animals of all ages have been filled with formalin, frozen, and cut into one-inch sections which were examined for structural make-up and evidences of abnormality, and photographed. The number of gross sections cut from each udder was dependent to a considerable extent on its size and varied from 6 to 46 with an average of 18, a total of more than 7,500 sections. In many cases one-half of the udder was used for the above studies, and the other half was sent to a pathologist who removed samples of the tissue for histological examination. It is particularly noteworthy that in the udders examined no growths or tissue changes that appeared to be of a cancerous nature have been found, though with the technique used a very small growth might have escaped notice.

Cancer usually does not make its appearance either in the human or in other species until after middle life. It is desirable, therefore, that consideration be given to the age of the 313 cows from which the udders included in this study were obtained. It is recognized that a distribution of ages of dairy cows at the time of death does not give a true picture of their normal life span, since most of them are disposed of when their reproductive functions become inactive; when they acquire some communicable disease, such as tuberculosis; or when they otherwise become incapacitated for satisfactory production or for reproduction. A study of the ages of a random sample from nearly 500,000 cows in Dairy Herd Improvement Associations shows that only 4 per cent of the total number were over 10 years, only 1 per cent were over 12 and only one-tenth of 1 per cent were over 15 years of age. It is true that many cows are not slaughtered but die as a result of injury or

disease. On the other hand cows rarely die directly as a result of "old age" and no data are available to show what the actual life span of the cow would be if it were undisturbed by economic factors.

There are a number of lines of reasoning by means of which a purely arbitrary basis can be set up for comparing the life span of the cow with that of the human. At best, however, any basis so set up is only an approximation. The period of fertility might be used as a basis for determining age equivalents. The beginning of the fertile period is fairly definite in each case, but in attempting to establish the age at which cessation of activity in the reproductive functions occurs, one is confronted with a physiological difference which establishes a rather definite termination in the woman but not in the cow. Then too, one might compare the life expectancy at birth, which is approximately 65 years for human white females,[1] with an arbitrary assumed expectancy of 13 years for the cow. On this basis the life span would be 5 times as great for the woman as for the cow and the woman would be 5 times as old as the cow at any given age. This would make the cow of 8 years equivalent in age to the woman of 40.

The following discussion illustrates another basis for estimating age equivalents in the woman and in the cow. In most cases the human female is capable of producing offspring at from 14 to 16 years of age and a woman is usually considered senile if she lives to the age of 80 years. On the other hand a cow usually is capable of delivering offspring at from 1½ to 2 years of age and if she lives to be 18 years of age she is considered to be very old—perhaps comparable in age to the woman at 80. Using 16 years and 2 years respectively as points representing sexual maturity in the woman and in the cow, the subsequent span of life is 64 years in the woman and 16 years in the cow, if 80 years and 18 years respectively are accepted as representing senility. The resulting age equivalents for the woman and for the cow, together with the distribution of the ages at death of the 313 cows whose udders were studied, are given in table 1.

On this basis, as well as on the second one mentioned for comparing life cycles, the cow of 8 years would be equivalent in age to the woman of 40 years. It may, therefore, be significant that 98 of the 313 cows of lactating age from which udders were secured, or 31 per cent, were cows that were over 8 years of age. It appears that a sufficient proportion of the cows studied were past middle life at the time of death to place them in that part of the life cycle in which mammary cancer may be expected to make its appearance in species that are susceptible, yet growths that appeared to be of a cancerous nature were not found in any of the udders.

As a matter of fact, the findings of numerous investigators show that cancer is virtually non-existent in the udder of the cow. According to

[1] Statistical Bulletin, Metropolitan Life Insurance Company, Vol. 20, No. 8, August 1939, p. 2.

TABLE 1

*Age equivalents for woman and for cow, and distribution of ages at death of 313 cows whose udders were studied*

| Approximate equivalence in age | | Age distribution of the 313 cows whose udders were included in this study | |
|---|---|---|---|
| Woman | Cow | Age | Number of cows |
| *Years* | *Years* | *Years* | |
| 16[a] | 2[a] | 2 and under 3 | 23 |
| 20 | 3 | 3 " " 4 | 49 |
| 24 | 4 | 4 " " 5 | 42 |
| 28 | 5 | 5 " " 6 | 27 |
| 32 | 6 | 6 " " 7 | 39 |
| 36 | 7 | 7 " " 8 | 35 |
| 40 | 8 | 8 " " 9 | 24 |
| 44 | 9 | 9 " " 10 | 18 |
| 48 | 10 | 10 " " 11 | 23 |
| 52 | 11 | 11 " " 12 | 20 |
| 56 | 12 | 12 " " 13 | 8 |
| 60 | 13 | 13 " " 14 | 2 |
| 64 | 14 | 14 " " 15 | |
| 68 | 15 | 15 " " 16 | 2 |
| 72 | 16 | 16 " " 17 | |
| 76 | 17 | 17 " " 18 | |
| 80[b] | 18[b] | Over 18 | 1 |

[a] Approximate age of sexual maturity.
[b] Approximate age of senility.

Trotter (3) "Cancer of the mamma and uterus is of frequent occurrence in the human female, but in the 300 cases here recorded as occurring in bovines quite a marked difference is noted, none being found in the mamma, and only one in the uterus—a carcinoma." Trout (4) stated that carcinoma of the udder of the milk cow is practically unknown. Drabble (1) quoted Doctor Dodd as referring to "the rarity of cancer in the udder of the cow." Feldman (2), in reporting on nearly 13 million bovines slaughtered subject to meat inspection by the Bureau of Animal Industry in 1930, showed that approximately 1,300 cases of tumorous growths were found but did not indicate that any occurred in the mammary glands. In fact Creech,[2] who reviewed the laboratory findings recorded in a large number of bovine tumors observed in connection with meat inspection activities involving the slaughter of many millions of cattle, over a period of years, has concluded that cancerous growths in the bovine mammary gland are very rare, and that those that have been found apparently originated from carcinoma of the skin and invaded the udder from that source.

It is difficult to understand why cancer, a disease of such high incidence in the mammary glands of humans and in other species should be so nearly non-existent in the cow's udder—particularly in view of the fact that that organ is so highly developed functionally, is often of enormous size, and

[2] Creech, G. T. Veterinarian, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture. Correspondence and conversation.

may be subject to friction, irritation and bruises. Nevertheless it is gratifying to know that the gland in which one of the most important foods used for human consumption is produced, is practically free from cancerous growths. This is particularly true in the light of recent investigations reported by Bittner (5), which show that in mice the incidence of breast cancer may be increased in the young of low cancer strains that are allowed to nurse females of a high cancer strain; or may be decreased in the young of high cancer stock that are allowed to nurse females of a low cancer strain. These results are interpreted by Bittner (5) as indicating that "the extrachromosomal or maternal influence observed in 'breast tumor crosses may be due to a breast cancer-producing influence' transmitted in the milk of breast tumor stock mothers."

## REFERENCES

(1) DRABBLE, J. Skin cancer in cattle. Australian Vet. J., 5: 2, 71–76. 1929.
(2) FELDMAN, WM. H. Neoplasms in domesticated animals. W. B. Saunders Co., Philadelphia and London. 1932.
(3) TROTTER, A. M. Malignant diseases in bovines. J. Comp. Path. (London), 24: 1, 1–20. 1911.
(4) TROUT, HUGH H. Treatment of carcinoma of the breast. J. Am. M. Ass., 3: 6, 489–492. 1938.
(5) BITTNER, JOHN J. Breast cancer and mother's milk. J. Heredity, 28: 11, 363–365. 1937.

# HEMOLYTIC STREPTOCOCCI IN RAW MARKET MILK

J. B. GUNNISON, M. P. LUXEN, M. S. MARSHALL AND B. Q. ENGLE
*Department of Bacteriology, University of California Medical School, and the City and County Department of Public Health, San Francisco, California*

The Lancefield technic (1) for the serologic grouping of hemolytic streptococci has furnished, for the first time, a method of detecting significant differences within this group of organisms. This procedure has been used to study streptococci of bovine origin by Plastridge and Hartsell (2), Stableforth (3), Stewart (4) and Edwards (5) among others. Sherman and Niven (6) and Valentine (7) have applied the precipitin method to surveys of the hemolytic streptococci which normally occur in market milk from the state of New York.

The samples tested in the present study were obtained through the kind cooperation of Dr. J. C. Geiger, Director of Public Health of the City and County of San Francisco. All were of raw milk or cream which was to be pasteurized and distributed as a grade A product. During a period of three months, one sample from each of 444 different shippers to this city was tested. Each milk specimen was from a pool of the morning milking from a given herd of 10 to 600 cows. Cream samples were from the pooled lot skimmed at a given plant or station for that morning and hence each was derived from many dairy farms. Samples were collected as a rule from ten gallon shipping tanks. The milk was mixed with a sterile agitator and approximately 200 cc. were transferred to a sterile eight ounce bottle by means of a sterile glass tube. The specimens were kept on ice and were tested within 3 to 10 hours after collection.

Each sample was placed in beef heart infusion agar containing five per cent sheep blood. After 24 to 48 hours at 37° C. colonies which showed any degree of hemolysis but no green discoloration were transferred to beef heart infusion broth containing particles of meat.

All cultures were grouped by the Lancefield precipitin technic using one tube containing 0.1 cc. of antigen, 0.3 cc. of saline and 0.1 cc. of serum for each antiserum tested. Many strains were tested also by the microscopic precipitin method of Brown (8). Tests were made for the fermentation of sorbitol, trehalose, salicin, lactose, raffinose, glycerin and mannite; for the hydrolysis of sodium hippurate and of starch; for the curdling of milk; for the production of soluble hemolysin and for the formation of double zones of hemolysis in blood agar. The procedures are given in detail in another paper (9).

## RESULTS

Hemolytic streptococci were found in 134 of 444 samples of raw market milk from as many different dairy farms and in five of nineteen lots of

Received for publication November 22, 1939.

cream. The proportion of samples in which such streptococci were found was slightly higher among samples collected at country skimming and cooling stations than among those from city pasteurization plants. The number of positive specimens from different plants varied from one to eight per twelve samples.

The counts of hemolytic streptococcus colonies from the milk were low with but few exceptions. Most of the samples yielded less than 300 hemolytic streptococci per cubic centimeter, very few showed more than 1,000 per cubic centimeter and none contained over 3,000 per cubic centimeter.

Serologic tests showed that streptococci of group B were virtually always present in positive samples. However, organisms of every group except F were found. Seven samples contained streptococci of more than one group. As shown in table 1, the distribution of the various groups was as follows: group A in three samples, group B in 130, group C in three, group D in two, group E in two, group G in six and group H in one. Four samples contained streptococci which failed to give definite reactions with any antiserum although they showed weak precipitation with serums of group C and G after standing over night.

Biochemical tests as well as serological reactions were carried out with

TABLE 1

*Serologic grouping of hemolytic streptococci from raw milk and cream samples*

| Source | No. tested | No. in which strept. found | Number in which Streptococci found of group: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | F | G | H | ? |
| MILK | | | | | | | | | | | |
| Pasteurization plants, San Francisco: | | | | | | | | | | | |
| I | 33 | 18 | 1 | 18 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| II | 20 | 9 | 0 | 9 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| III | 45 | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IV | 12 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| V | 12 | 4 | 0 | 3 | 1* | 0 | 0 | 0 | 0 | 0 | 0 |
| VI–XIX (Plants with less than 10 shippers) | 52 | 12 | 0 | 11 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 174 | 50 | 1 | 48 | 2 | 1 | 2 | 0 | 0 | 0 | 0 |
| Country skimming and cooling stations: | | | | | | | | | | | |
| XX | 88 | 32 | 0 | 28 | 0 | 0 | 0 | 0 | 6 | 1 | 1 |
| XXI | 26 | 16 | 1 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| XXII | 22 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| XXIII | 50 | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| XXIV | 78 | 26 | 1 | 24 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| XXV | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 270 | 84 | 2 | 77 | 3 | 1 | 0 | 0 | 6 | 1 | 4 |
| CREAM | 19 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total—All samples | 463 | 139 | 3 | 130 | 3 | 2 | 2 | 0 | 6 | 1 | 4 |

* "Animal pyogenes."

293 cultures from 139 samples of milk or cream. The results, discussed in more detail in another paper (9), are summarized in table 2.

TABLE 2

*Reactions of hemolytic streptococci from milk*

| Serologic group | No. of strains tested | No. of samples from which derived | Acid produced from | | | | | | | Milk curdled | Starch hydrolyzed | Sodium hippurate hydrolyzed | Soluble hemolysin formed | Double zones of hemolysis formed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sorbitol | Trehalose | Salicin | Lactose | Raffinose | Glycerin | Mannite | | | | | |
| A | 3 | 2 | − | + | + | + | − | − | − | − | 2+ | − | + | − |
| A | 3 | 1 | − | − | − | − | − | − | − | − | 1− | + | + | − |
| B | 165 | 99 | − | 161+<br>4− | − | + | − | 7+<br>158− | − | + | − | + | + | 66+<br>99− |
| B | 92 | 47 | − | + | + | + | − | 10+<br>82− | − | + | − | + | + | 67+<br>25− |
| C | 6 | 1 | + | − | + | + | − | − | − | − | + | − | + | − |
| C | 3 | 2 | − | + | + | + | − | − | − | + | − | − | + | − |
| D | 2 | 2 | − | − | − | + | − | − | − | + | − | + | − | − |
| E | 4 | 2 | + | + | + | + | − | − | + | − | − | − | + | − |
| G | 8 | 6 | − | − | − | + | − | − | − | − | − | − | + | − |
| H | 1 | 1 | − | − | − | + | − | − | − | + | − | − | − | − |
| ? | 7 | 4 | − | − | − | + | 2+<br>5− | − | − | 6+<br>1− | − | − | + | − |

The reactions of 257 strains of group B were, in general, uniform. None fermented sorbitol, raffinose, or mannite; all produced acid from lactose and all but four fermented trehalose. Seventeen cultures irregularly produced a slight amount of acid from glycerin. Salicin was fermented by 92 strains and was unchanged by 165 of them. Milk was curdled and sodium hippurate was hydrolyzed by all and none attacked starch. All showed at least a trace of soluble hemolysin, 76 gave nearly complete hemolysis and 27 completely hemolyzed red blood cells.

Slightly over half of the members of group B produced double zones of hemolysis in rabbit blood agar plates. After 48 hours at 37° C. and 24 hours in the refrigerator these cultures showed a narrow completely clear zone of hemolysis immediately adjacent to the colony surrounded by a narrower ring of unhemolyzed cells and then by a zone of partial hemolysis somewhat wider than the innermost zone. This double zone phenomenon was observed more often among the salicin fermenting strains (67 of 92) than among those which did not attack this sugar (66 of 165). In sheep blood agar plates, incubated at 37° C. for 48 hours but not refrigerated, double zones were never observed. Under these conditions, about a fourth of the group B streptococci formed clear wide zones of hemolysis but the majority produced narrow areas in which some intact corpuscles were present.

Some samples contained large numbers of streptococci which produced a greenish discoloration of the α type around surface colonies but which gave typical colorless narrow zones of β hemolysis in deep colonies. Twenty-four of these green-producing colonies were found to be identical both serologically and biochemically with the cultures which showed only β hemolysis.

Usually biochemical tests were made of only one strain of group B per sample, but from 61 samples from two to ten colonies were studied. All from a given specimen gave identical reactions in 39 instances. The only variations found in the cultures in which reactions were not identical were in the fermentation of salicin and in the formation of double zones of hemolysis. From nine samples of milk both salicin-fermenting and salicin-negative colonies were isolated, but these cultures were identical in other respects. Cultures which varied only in their ability to form double zones were obtained from eight samples. In five samples some of the colonies failed to ferment salicin and to show double zones and others possessed both of these properties. Whether such milk actually contained two distinct strains of group B streptococci or whether these differences were due to variability of the cultures is not known.

Only two samples of milk contained typical *Streptococcus pyogenes* of group A. Group B organisms were also present in one of these samples. One specimen contained atypical streptococci which gave strong precipitation with group A antiserum but which hydrolyzed sodium hippurate and failed to ferment any of the sugars tested. These atypical cultures were repeatedly streaked out and repurified but all colonies still gave the same peculiar reactions. No streptococci of other groups could be found in this sample.

The so-called "animal pyogenes" type of group C which ferments sorbitol but not trehalose and which hydrolyzes starch was found in only one lot of milk. The "human" type of group C fermenting trehalose but not sorbitol was isolated from two samples.

Hemolytic cultures of group D which were encountered twice could not be assigned to a species by the tests used. These were not resistant to heating at 60° C. for 30 minutes.

Group E streptococci were found in milk from two dairy farms. The cultural reactions agreed closely with those of the few cultures which have been described. They showed a wide zone of clear cut hemolysis on blood plates and produced potent soluble hemolysin. These were resistant to 60° C. for 30 minutes.

Group G organisms were found in six of the samples. All of these cultures produced acid from lactose but failed to ferment the other sugars used. They were strongly hemolytic both on plates and in the soluble hemolysin test and did not hydrolyze starch or sodium hippurate. The colonies were

not of the "minute" type. A single sample yielded a culture of group H which resembled the group G organisms in every respect except that it failed to produce soluble hemolysin under the conditions of these tests.

## DISCUSSION

No relationship was evident between the number and kinds of hemolytic streptococci found in the milk and factors such as the sanitary rating of the dairy farm or its geographical location, the distance of shipping or the number or breed of cattle in the herd. Although the country skimming and cooling stations showed a slightly higher proportion of positive samples than the city pasteurization plants, the difference was not significant.

The method used in this survey does not give a true index of the actual number of group B streptococci present because only β hemolytic organisms were considered. It has been established by Stableforth (10) and others that members of this group may be entirely non-hemolytic or may give a greenish discoloration of the α type. If such colonies had been studied, the proportion of positive samples in all probability would have been higher. Furthermore, non-hemolytic members of groups C, D, G and H, which were not taken into account here, have been described by several investigators.

The incidence of hemolytic streptococci was lower than that reported by others. Valentine (7) obtained hemolytic streptococci from 138 of 244 samples of grade A and B milk. Not more than 30 samples among the 463 in the present series contained streptococci which produced wide zones of hemolysis, whereas Sherman and Niven (6) found 19 such samples among 68 tested or approximately four times as many. They stated that if they had considered narrow zone cultures they would have obtained group B streptococci from nearly every sample, whereas these organisms were found in only about a third of the specimens studied here.

In this survey eight kinds of identifiable hemolytic streptococci were isolated; namely, group A, group B, both the human and animal types of group C, group D, group E, group G and group H. Sherman and Niven (6) found only two kinds of hemolytic streptococci in raw milk; namely group B and the animal type of group C. Valentine (7) found group B, both the animal and human types of group C and group D. The fact that these investigators found fewer varieties might be due to their smaller series of samples or to the presence of larger numbers of other bacteria in the milk. The milk studied here showed extremely low total plate counts and it has frequently been observed that streptococci, if present, can be detected more readily in such milk.

As far as could be determined this is the first time that serologically identified streptococci of groups G and H have been reported in raw milk. Hemolytic group D cultures have been isolated by others from cheese (1)

and from pasteurized milk (6). They are probably present, at least in small numbers, in most raw milk and yet they were found in only two samples in this series and in only seven of Valentine's samples.

Streptococci of the animal type of group C were found in only one sample. Sherman and Niven (6) did not state how often they isolated this organism; but inasmuch as they selected only two or three colonies per sample and described eleven such cultures, the "animal pyogenes" must have been found in at least four or five of their 68 specimens. Valentine (7) reported that this sorbitol fermenting type of group C was found in 57 of 120 samples and hence was more common than group B which was found in only 13 of her specimens.

The group A, G and H strains with atypical cultural reactions are noteworthy because they illustrate the fact that cultures are encountered which can not be identified by any means other than the serologic test. The occurrence of group A cultures which hydrolyze sodium hippurate was of especial interest for such streptococci seem to be rare.

It is not known whether or not all cultures of group B should be given the name *Streptococcus agalactiae* (or *mastitidis*) or whether the group contains more than one species. Stableforth (3), Lancefield (11) and Stewart (4) have found from three to five serologic types within the group. The groups may be subdivided into three varieties according to the reactions on lactose and salicin as proposed by Brown (12), but there is no apparent correlation of the fermentation reactions with the serologic types. The salicin +, lactose + variety has been obtained frequently from both bovine and human sources. Thus far the salicin +, lactose – type has never been isolated and identified by serologic tests from milk. The salicin –, lactose + variety has been found principally in milk but six strains isolated from the throats of children by Plummer (13) were identified as group B by Brown (12) and one culture was obtained from urine by the present writers (9). The available evidence suggests that this variety may be of bovine origin. Frost, Gumm and Thomas (14) gave the name *Streptococcus asalignus* to non-salicin fermenting streptococci from milk. Although these were not classified by the precipitin test it is likely, as Sherman (15) and Brown (12) have pointed out, that they belonged to group B. It does not seem justifiable to regard this variety as a species distinct from *Streptococcus agalactiae* merely because it fails to ferment salicin and is rarely found in human beings. It is not known how stable the streptococci are with regard to their ability to attack salicin and it seems quite probable that this may be a variable characteristic.

Both the salicin +, lactose + and the salicin –, lactose + varieties of group B produce mastitis in cattle under natural conditions (12). Little (16) found that the salicin +, lactose – strains from human infections produced mastitis when experimentally introduced. Hence, even if these prove to be

exclusively of human origin, it seems possible that they may occasionally be transferred to milk in the same manner as *Streptococcus pyogenes* although this has not been reported as yet.

No cultures resembling the *Streptococcus pseudo-agalactiae* of Plastridge and Hartsell (2) were encountered. They described this species as differing from *Streptococcus agalactiae* only in its usual failure to curdle milk, its occasional reduction of methylene blue and its slight precipitin reactions with antiserums of both groups B and C but strong reactions with its homologous antiserum. It seems doubtful that this variety should be assigned to a separate species.

Hence the decision as to the further subdivision of group B must await further investigation. It would be just as logical to divide it on the basis of double zone formation as on the basis of reactions with salicin and lactose unless a sound serological, cultural and epidemiologic correlation can be shown. In the present state of confusion an effort toward unification rather than hair-splitting subdivision would seem to be indicated.

Brown (12) has stated that all streptococci which form double zones of hemolysis in rabbit blood agar belong to group B, but that there are a few strains of group B which do not form double zones. In the present study and in a previous one (9) no double zone streptococci were found which belonged to groups other than group B. However, nearly half of the group B cultures failed to show this phenomenon although the tests were made repeatedly under the same conditions as those of Brown. Hence group B streptococci could not be identified by this means.

Among the cultural reactions employed in this study those which seemed of value as differential criteria were the fermentation of sorbitol, trehalose and salicin and especially the hydrolysis of sodium hippurate. The determination of the final pH of cultures in glucose broth was used in the earlier part of the survey but was later abandoned. It did not seem to be sufficiently reliable inasmuch as there were strains in all groups which reduced the pH to the 4.8 to 4.6 range. There are, of course, many other valuable biochemical and physiologic tests which were not employed herein, but which would have been indispensable in the absence of the group precipitin method of classification.

Both Brown (17) and Sherman and Niven (6) have suggested that the fibrinolytic test of Tillett and Garner (18) would be an aid in studying milk streptococci. By this test organisms of group A and the human types of groups C and G which dissolve human fibrin can be differentiated from all other streptococci thus far studied. These three groups unfortunately are often impossible to distinguish from each other by biochemical means. Sherman's hope that glycerin and starch tests might serve this purpose was not borne out by subsequent studies (9). It would seem then that the fibrinolytic test which is even more time-consuming and technically difficult than the serologic procedure fails to give as definite an identification.

Hence it is advocated that, for studies of milk in which it is necessary to identify hemolytic streptococci, the serologic method should be used as soon as serum is available commercially. The precipitin grouping must be supplemented by fermentation tests with sorbitol to differentiate between animal and human group C types. In order to identify the various species of group D the tests outlined by Sherman (15) must be used. These include the ability to grow at 45° C. or at 10° C.; growth in the presence of 6.5 per cent bile blood agar; the production of ammonia and the heat resistance at 60° C. for 30 minutes. Undoubtedly additional biochemical and physiological methods will be added to this list as further information is obtained.

The fact that high grade milk produced under rigid inspection and, so far as the plate count is concerned, above the standard set for certified milk, may contain hemolytic streptococci of human origin is a strong argument for universal pasteurization even though the number of instances in which such organisms are found is extremely low. In this city all milk including the certified grade must be pasteurized.

## SUMMARY

A total of 444 samples of raw market milk from as many different dairy farms was examined for the presence of hemolytic streptococci of both wide-zone and narrow-zone types. Such streptococci were found in 134 of these samples and were distributed among the Lancefield serologic groups as follows: group A in three samples, group B in 125, group C of the animal type in one, group C of the human type in two, group D in two, group E in two, group G in six and group H in one. Only four samples contained streptococci which could not be assigned to a group. Nineteen samples of raw cream were tested and group B streptococci were found in five of them. Approximately half of the group B streptococci produced double zones of hemolysis in rabbit blood agar.

## REFERENCES

(1) LANCEFIELD, R. C. A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med., 57: 571–595. 1933.

(2) PLASTRIDGE, W. N., AND HARTSELL, S. E. Biochemical and serological characteristics of streptococci of bovine origin. J. Inf. Dis., 61: 110–121. 1937.

(3) STABLEFORTH, A. W. Serological types of Str. agalactiae (streptococcus group B) in this and other countries. J. Path. Bact., 45: 263–277. 1937.

(4) STEWART, D. F. Group classification and serologic typing of streptococci associated with bovine mastitis in Australia. J. Path. Bact., 45: 279–293. 1937.

(5) EDWARDS, P. R. The differentiation of hemolytic streptococci of human and animal origin by group precipitin tests. J. Bact., 27: 527–534. 1934.

(6) SHERMAN, J. M., AND NIVEN, C. F. Hemolytic streptococci of milk. J. Inf. Dis., 62: 190–201. 1938.

(7) VALENTINE, E. Personal communication. Reported in Grade A milk survey conducted by Dr. W. L. Carr in New York City. 1938.

(8) BROWN, J. H. A simplified method for grouping hemolytic streptococci by the precipitin reaction. J. Am. M. Ass., **111**: 5, 310–311. 1938.

(9) GUNNISON, J. B., LUXEN, M. P., CUMMINGS, J. R., AND MARSHALL, M. S. The differentiation of hemolytic streptococci from various sources by the group precipitin reaction and by biochemical tests. J. Bact. (in press).

(10) STABLEFORTH, A. W. Studies on bovine mastitis. VII. The serological characters of mastitis streptococci. J. Comp. Path. Therap., **45**: 185–211. 1932.

(11) LANCEFIELD, R. C. A serological differentiation of specific types of bovine hemolytic streptococci (group B). J. Exp. Med., **59**: 441–458. 1934.

(12) BROWN, J. H. Double-zone beta-hemolytic streptococci; their cultural characteristics, serological grouping, occurrence, and pathogenic significance. J. Bact., **37**: 133–144. 1939.

(13) PLUMMER, H. The fermentation of sorbitol and trehalose by haemolytic streptococci from various sources. J. Bact., **27**: 465–472. 1934.

(14) FROST, W. D., GUMM, M., AND THOMAS, R. C. Types of hemolytic streptococci in certified milk. J. Inf. Dis., **40**: 699–705. 1927.

(15) SHERMAN, J. M. Streptococci. Bact. Rev., **1**: 3–97. 1937.

(16) LITTLE, R. B. The significance of human double zone beta hemolytic streptococci in the udder of the cow. J. Exp. Med., **68**: 905–911. 1938.

(17) BROWN, J. H. Significance of the hemolytic streptococci found in milk. Cornell Vet., **27**: 110–121. 1937.

(18) TILLETT, W. S., AND GARNER, R. L. The fibrinolytic activity of hemolytic streptococci. J. Exp. Med., **58**: 485–502. 1933.

# STUDY OF DAIRY CLEANING PROBLEMS I. FILMS AND DEPOSITS ON HOT-MILK EQUIPMENT

J. J. JOHNSON AND C. T. ROLAND*
*Sealtest, Inc., Research Laboratory, Baltimore, Maryland*

The removal of films or deposits from dairy equipment used in the heating and holding of milk products often presents a serious problem. Parker and Johnson (1) early directed attention to the practical and scientific aspects of this problem. They defined *milk film* as the deposit which forms on metal heat-transfer surfaces due to the precipitating action of the heat alone. *Milkstone* was defined as the product resulting from the reaction between the milk film as described and the chemical constituents of the water supply and alkaline detergents applied or their end products.

In commercial operations where milk products are heated, particularly above 140° F., by metal heat-transfer surfaces, there appears to be a daily film formation of some extent depending on the thermal differential and the type of equipment. This may vary from an extremely thin, transparent or translucent film appearing as a bluish or brown discoloration when viewed from an angle, on heater plates or tubes, to a heavy cheese-like blanket of milk solids on batch pasteurizing vats in which relatively rapid heating is accomplished by means of a high jacket temperature. Before an effort was made to determine the most suitable detergents or cleaning methods for hot-milk equipment, it was deemed advisable to study further the mechanism of the film formation on metal heat-transfer surfaces and to account, if possible, for some of the differences observed.

## EXPERIMENTS

A simple apparatus was devised by means of which milk could be heated continuously under controlled conditions. The apparatus consisted of a 9-inch length of 2-inch stainless steel tubing stoppered on both ends with rubber stoppers. One stopper contained inlet and outlet tubes of 5 mm. I. D. glass tubing and a thermometer, of which only the bulb was extended on the inside. The inlet tube extended to within one inch of the bottom stopper. The outlet tube was flush with the inner surface of the top-retaining stopper. This assembly was placed vertically in an agitated, gas-heated water bath. The raw milk of 4 per cent fat content, forewarmed to 85° F., was allowed to flow through the tube by gravity, being drawn off at the desired temperature through regulating the flow by means of a stop-cock on the inlet tube.

Received for publication November 24, 1939.

* Now with Calgon, Inc., 300 Ross Street, Pittsburgh, Pennsylvania.

*Trial 1.* In this experiment 7 liters of milk, forewarmed to 85° F., were heated to 143° F. in the tube, maintaining the jacket at 180° F. The milk was cooled continuously to 85° F. after passing through the tube and was recirculated continuously. The milk was collected in a graduate and the rate of flow was measured. After the initial quantity of milk in the tube reached 143° F., the time required to heat the first liter portion was 6 minutes. The heating time increased gradually because of film formation on the heating surface until that for the thirteenth liter (the last one) was 12 minutes. At the end of the run the milk remaining in the tube was poured out and a liter of cold water passed through the tube to rinse the deposit. The deposit consisted of milk solids in the form of small craters of cheese-like consistency. These completely covered the upper part of the tube and graded off to a sparse covering of the lower part. Their diameters were about 0.5 to 1.0 mm. When the soft material was scraped off, there was found to be a fairly strong bond between the metal and the milk solids. The tube was cleaned with hot washing powder solution and powdered pumice in preparation for the next trial.

*Trial 2.* In this experiment 7 liters of raw milk were heated to 143° F. with the jacket at 180° F. This milk was drawn off in liter portions but not recirculated. The time required for heating liter portions varied from 7 minutes for the first to 10 minutes for the seventh. The tube was rinsed with a liter of cold water and opened for inspection. The heating surface was quite evenly covered with the small crater-like particles, many of which contained gas bubbles. Furthermore, it was observed during this run that considerable gas was expelled from the milk and appeared at the outlet tube. These observations suggested that the dissolved gases in the milk might play an important part in the formation of this type of heat-precipitation. The deposit was scraped out and analyzed with the following results:

| | |
|---|---|
| Fat | 51.53% |
| Protein ($N \times 6.38$) | 35.32 |
| Ash | 4.77 |
| CaO | 2.28 |
| $P_2O_5$ | 2.10 |

The tube was cleaned with hot washing powder solution and powdered pumice. Vigorous scouring was required to eliminate the last traces of the deposit which appeared as a bluish discoloration of the surface, discernible only after the milk solids had been removed.

*Trial 3.* In this experiment 7 liters of milk were held under 15–16 mm. (Hg) vacuum at a temperature of about 55° F. for 2 hours. The temperature was elevated then to about 70° F. and the milk allowed to boil gently for 10 minutes. The vacuum was broken then, the milk warmed

to 85° F. and 7 liters heated in the tube, as in previous tests, to 143° F. with a jacket temperature of 180° F. The rate of heat transfer during this run did not vary significantly, 5 minutes being required to heat the first as well as the seventh liter. When the tube was opened, it appeared that there was no deposit whatsoever on the surface; but after rinsing with distilled water and drying in air, it became apparent that a bluish discoloration covered the heat-transfer surface of the tube. When the surface was viewed as nearly as possible at right angles, it appeared as a very thin, translucent film.

*Trial 4.* In this experiment milk was deaerated as in Trial 3 and heated in the tube to 143° F. with the jacket at 200° F. The first liter through required 3 minutes, the seventh 4 minutes. When the tube was rinsed and opened, a few very small craters of cheese-like consistency were present and the whole heat-transfer surface was covered with the bluish discoloration as previously observed. Observation from a nearly perpendicular position showed the surface to be covered with a thin, dense whitish film.

*Trial 5.* In this experiment 7 liters of milk were deareated continuously by spraying the milk into a vacuum chamber. The milk, heated to 115° F., was drawn into a flask under 60 mm. (Hg) vacuum. The treatment required about 1 hour. Seven liters of the milk were heated as in previous experiments, this time to 170° F. with the jacket at 200° F. The first liter through required 3½ minutes and the seventh 5 minutes. After rinsing with a liter of cold water, the tube was opened. There were none of the precipitated milk solids on the heat-transfer surface. It had only the film appearing as a bluish discoloration when the tube was looked into and as a dense whitish translucent coating when viewed perpendicularly. There appeared to be more of the metallic-like film produced in this test than in the previous ones.

*Trial 6.* As a control to the previous experiment, untreated milk was heated in the tube to 170° F. with the jacket at 200° F. Only 5 liters were run through. The first required 8 minutes, the second 10 minutes, the third 13½ minutes, the fourth 17½ minutes and the fifth 21½ minutes. When the tube was rinsed and opened, a heavy blanket of the cheese-like milk solids was found deposited on the heat-transfer surface.

*Trial 7.* A practical test was made of the principle of high velocity flow against the metal heat-transfer surface as a means of eliminating the heat-precipitation of cheese-like solids due to the action of gas bubbles as previously described. An experimental procedure was being used in the technological laboratory whereby a 40-gallon batch of cold skimmilk was

heated and pasteurized at 203° F. by means of a 60-gallon stainless steel spray-type vat. This procedure was time-consuming and resulted in excessive deposits of milk solids even when low thermal differentials were used. In this test the skimmilk contained in the spray vat at 58° F. was circulated by means of a centrifugal pump through a box-tube heater, containing 6 stainless steel tubes one and one half inches × 6½ feet, at the rate of 67 g.p.m. or approximate average velocity of 5 feet per second. The temperature of the steam-heated water jacket of the heater at the beginning of the run was 180° F. and was gradually increased to 210° F. at the finish. To heat the 40 gallons of skimmilk from 58° F. to 203° F. required 12 minutes. At the end of the operation the temperature in the heater jacket was dropped rapidly and the circulation stopped. After draining and flushing the tubes with cold water an examination disclosed that there were no cheese-like solids on the heat-transfer surfaces. After the tubes had dried, some of the bluish discoloration or film was found in the tubes. There was surprisingly little of this, however, in comparison with that found in the laboratory experiments previously described. The hot milk was held in the spray vat for 30 minutes at 203° F., maintaining a 1°–2° higher temperature in the heating jacket, after which the milk was cooled and drawn off. The milk was rinsed off with cold water and the vat examined. Along the upper sides was found a trace of the film or discoloration. Cheese-like solids, however, were entirely absent.

## DISCUSSION

These tests have shown that 2 types of film may be produced on metal heat-transfer surfaces during the processing of milk. One is essentially cheese-like in composition and is believed to result from the super-heating and partial dehydration of the milk films on the surface of the gas bubbles which, in being expelled from the milk, cling to the metal heat-transfer surface. This is the type of film or deposit commonly found in jacketed vats or pans used for heating milk products. Were it not for the sweeping action of high velocity flow in tubular or plate heaters or the action of agitator blades in other types of heaters, it might be expected that more trouble would be encountered with this type of film.

Davies (2) observes the formation of particles, apparently of cheese-like consistency, on the surface of sterilized milk bottles. He attributes this to the precipitation of the proteins because of their concentration at the air/liquid interface of gas bubbles. He states also that slight heat coagulation on hot surfaces is associated with the foaming of fresh milk on boiling and that such coatings have a serious effect on the efficiency of heat exchange in pasteurizing or vacuum pan work.

The other type of film which is produced concomitantly with the cheese-like film or independent of it, according to conditions, is believed to consist

of calcium and magnesium phosphates, citrates and possibly proteinates, precipitated by the heat-transfer surface. Because of the difficulties encountered in procuring a sample of this extremely thin film, no analysis was made. This metallic-like film or discoloration, while it may be almost insignificant in extent after one processing, is believed to be the real precursor of milkstone found on hot-milk equipment. The cheese-like film dissolves readily in alkaline washing solutions, as will be shown in a following report, leaving the metallic-like film or discoloration. This is probably often overlooked in commercial operations until successive processings result in a film of such extent that the presence of milkstone is recognized. From the data presented by Parker and Johnson (1) and Tuckey (3), it is apparent that there are wide variations in the final composition of milkstone from commercial operations depending on such factors as daily cleaning efficiency, type of washing solution, type and hardness of water, and, as indicated in this work, the processing equipment and procedure.

## SUMMARY AND CONCLUSIONS

1. An apparatus and method was devised for studying the formation of heat-deposited milk films.

2. Two definite types of films were found: one of cheese-like consistency is the result of super-heating and partial dehydration of milk around gas bubbles formed at the heat-transfer surface; the other a thin dense metallic-like film or discoloration is produced concomitantly with the cheese-like deposit, or independent of it if conditions are such that gas bubbles do not form at the heat-transfer surface.

3. It is suggested that the accumulation of thin metallic-like films, due to incomplete cleaning or failure to recognize their presence, is the primary cause of milkstone formation on hot-milk equipment.

## REFERENCES

(1) Parker, M. E., and Johnson, A. H. Notes on the prevention and removal of milkstone. Proc. 23rd Ann. Conv. of the Int. Assoc. of Milk Dealers, Plant Sect., pp. 5–15. 1930.

(2) Davies, W. L. The chemistry of milk. Chap. XVI. The effect of heat on milk. pp. 345–6. D. Van Nostrand, New York. 1936.

(3) Tuckey, S. L. Milkstone formation, control and removal. Milk Plant Monthly, 20: 6, 46–47, 69. 1931.

# STUDY OF DAIRY CLEANING PROBLEMS. II. EFFECTIVENESS OF ALKALIES IN REMOVING HEAT-DEPOSITED MILK SOLIDS AND BUTTERFAT FILMS

J. J. JOHNSON AND C. T. ROLAND*
*Sealtest, Inc., Research Laboratory, Baltimore, Maryland*

Practical experience has shown that a satisfactory detergent for cleaning dairy equipment must be capable of dissolving or removing dried or heat-deposited milk products and be effective in "cutting" or emulsifying fat and grease. Other important attributes are non-corrosiveness to dairy metals and the ability to produce flocculent, non-adhering precipitates of calcium and magnesium salts in natural water or better still to maintain such salts in solution on the alkaline side.

## A. REMOVAL OF HEAT-DEPOSITED MILK SOLIDS

In order to determine the relative effectiveness of the common alkalies in dissolving and removing heat-deposited milk solids, a simple apparatus was devised by means of which a controlled and fairly reproducible deposit could be produced. It consisted of a 9-inch length of 2-inch stainless steel tubing closed at both ends with rubber stoppers. One stopper contained inlet and outlet tubes of 5 mm. I. D. glass tubing and a thermometer, of which only the bulb was extended on the inside. The inlet tube extended to within 1 inch of the bottom stopper. The outlet tube was flush with the inner surface of the retaining stopper. This assembly was placed vertically in an agitated gas-heated water bath held at 180° F.

In all tests 7-liter portions of mixed raw milk of 4 per cent fat content were forewarmed to 85° F. and allowed to flow by gravity through the heater tube. The milk was drawn off at a constant temperature of 143° F., regulating the flow by means of a stop-cock on the inlet tube. This process resulted in the formation of an evenly distributed coating of cheese-like milk solids on the heating surface of the tube. The nature of heat-deposited milk films was discussed in a previous study (1).

To test the ability of alkali solutions to dissolve and/or remove the milk solids, the tube was first rinsed with a liter of cold water to remove the adhering milk. From 10 per cent stock solutions of the alkalies a 500 cc. quantity of test solution of desired concentration was made by dilution with tap water. The solutions were circulated through the tube, using a 2- to 3-inch gravity head, for 30 minutes at 130° F. At the conclusion of the treatment the tube was opened and examined. The solution was rated *good* if the deposit was completely removed, *fair* if the deposit was softened com-

Received for publication November 24, 1939.

* Now with Calgon, Inc., 300 Ross Street, Pittsburgh, Pennsylvania.

TABLE 1

*Effectiveness of alkalies at various concentrations in removing heat-deposited milk solids*

| Alkali | Conc. | pH | Phenolphthalein alkalinity (as NaOH) | Methyl Orange alkalinity (as NaOH) | Deposit removal |
|---|---|---|---|---|---|
| | *Per cent* | | *Per cent* | *Per cent* | |
| Trisodium phosphate (com'l) | 0.100 | 11.4 | 0.012 | 0.026 | poor |
| " " " | 0.212 | 11.7 | 0.022 | 0.046 | fair |
| " " " | 0.300 | 11.8 | 0.041 | 0.075 | good |
| Sodium metasilicate (com'l) | 0.060 | 11.5 | 0.022 | 0.026 | poor |
| " " " | 0.100 | 11.7 | 0.036 | 0.040 | fair |
| " " " | 0.200 | 12.0 | 0.073 | 0.078 | good |
| Sodium carbonate anhyd. (C.P.) | 0.063 | 11.0 | 0.023 | 0.048 | poor |
| " " " " " | 0.100 | 11.1 | 0.040 | 0.074 | fair |
| " " " " " | 0.200 | 11.2 | 0.075 | 0.146 | good |
| Sodium hydroxide (C.P.) | 0.010 | 11.4 | 0.010 | 0.012 | poor |
| " " " " | 0.020 | 11.8 | 0.020 | 0.023 | fair |
| " " " " | 0.030 | 11.9 | 0.029 | 0.033 | fair |
| " " " " | 0.040 | 12.0 | 0.042 | 0.046 | good |

pletely and partially removed, and *poor* if the deposit was not affected or only slightly softened. Between each test the tube was scoured with powdered pumice. Alkalinity by titration and pH by the glass electrode were determined on each solution. The results of typical tests covering the range of effectiveness of the alkalies are shown in table 1.

It will be noted that the approximate minimum effective concentrations were as follows: trisodium phosphate 0.3 per cent, sodium metasilicate 0.2 per cent, sodium carbonate 0.2 per cent and sodium hydroxide 0.04 per cent. As a basis for comparison one solution of each alkali was formulated to produce a phenolphthalein alkalinity of about 0.02 per cent as NaOH. At this alkalinity trisodium phosphate and sodium hydroxide were more effective than sodium metasilicate and sodium carbonate. It is of interest to note that sodium carbonate was effective at a considerably lower pH value than the other alkalies. It should be pointed out that the complete removal of the cheese-like deposit by the alkalies studied did not result in chemically clean metal surfaces. The very slight metallic-like film or discoloration was always found to be present after the cheese-like deposit has been removed. It is believed that this fact is not generally recognized in commercial operations.

### B. REMOVAL OF BUTTERFAT FILMS

A quantity of dry, clean butterfat was prepared from sweet butter and dyed dark red with oil soluble red dye. New soft-glass culture tubes ($15 \times 150$ mm.) were washed with soap, rinsed. and air dried. The tubes were filled with the melted fat at 130° F., emptied immediately and allowed to drain for 5 minutes in an oven at 130° F. after which they were turned upright and held for an additional 5 minutes. This resulted in the formation of a fairly thin film of fat on the inside of the tube. Immediately after the final holding period the tubes were transferred to a constant temperature water bath at 130° F. where they were immersed to within 2 or 3 cm. of the top. Ten-cc. portions of the various detergent solutions at 130° F. were allowed to drop into the tubes from the wide end of a 10-cc. bulb-type pipette held centrally over the mouths of the tubes. After standing in the bath for 10 minutes the tubes were carefully removed and photographed. Each test was made in duplicate and every precaution was taken in making the tests and photographing the results to insure comparability. This test is a modification of Baker's procedure (2).

A series of tests were made, using pure sodium hydroxide, sodium carbonate (anhyd.), commercial sodium metasilicate, and trisodium phosphate. Tap-water solutions ranging from 0.05 per cent to 2.0 per cent were made from 10 per cent stock solutions of the alkalies. The photographic results are shown in plate 1. It should be pointed out that fat remaining on the glass surface appears as dark globules or patches. The darkness of

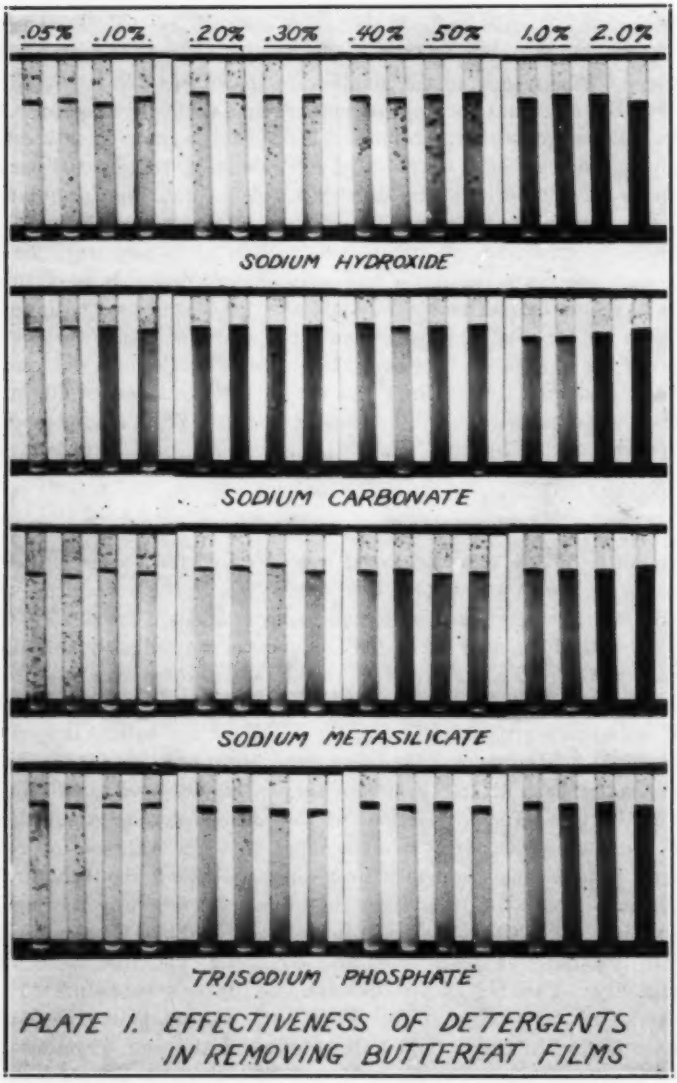



PLATE I. EFFECTIVENESS OF DETERGENTS IN REMOVING BUTTERFAT FILMS

the liquid indicates creaming or emulsification of the fat. It appears that sodium hydroxide alone was not effective at any concentration studied in the removal of butterfat films. This agrees with the experience of many plant operators who have observed poor rinsing or greasy bottles produced by a mechanical bottle washer using caustic soda alone as the detergent. In the range of concentrations generally used for equipment cleaning (0.1 per cent to 0.5 per cent), there seems to be very little difference in the effectiveness of the other three alkalies. The removal of the fat appears to be associated with a greater degree of emulsification in the case of sodium carbonate and sodium metasilicate than in the case of trisodium phosphate but the commercial significance of these small differences is questionable.

In order to study fat removal as related to bottle washing, tap-water solutions of 1.8 per cent sodium hydroxide and 0.2 per cent each of sodium carbonate, sodium metasilicate and trisodium phosphate were tested. Tap-water solutions of sodium hydroxide at 2 per cent with additions of 0.02 per cent, 0.1 per cent and 0.2 per cent of detergent A (a surface-active organic detergent) were tested also. Tap-water solutions of castile soap at 0.1 per cent and detergent A at 0.02 per cent and 0.2 per cent were included for comparison. The photographic results are shown in plate 2. The combination of sodium hydroxide and sodium carbonate did not produce as good fat removal as did the combinations with sodium metasilicate, trisodium phosphate and detergent A. Detergent A alone was very effective in removing the fat, although there was no evidence of emulsification as was the case with the alkalies.

There appears to be a fundamental difference between the activity of sodium metasilicate with sodium hydroxide and that of trisodium phosphate with sodium hydroxide. The metasilicate combination showed a marked "cream" whereas the phosphate combination held the fat at the surface in a more or less continuous fat phase. The latter was true also of the combination of sodium hydroxide and detergent A.

## SUMMARY AND CONCLUSIONS

1. The approximate minimum concentrations of alkalies effective in removing heat-deposited milk solids were as follows: trisodium phosphate 0.3 per cent, sodium metasilicate 0.2 per cent, sodium carbonate 0.2 per cent and sodium hydroxide 0.04 per cent.

2. At a phenolphthalein alkalinity of approximately 0.02 per cent (as NaOH) trisodium phosphate and sodium hydroxide were more effective than sodium metasilicate and sodium carbonate in removing heat-deposited milk solids. The minimum effective pH values were as follows: sodium carbonate 11.2, trisodium phosphate 11.8, sodium metasilicate 12.0 and sodium hydroxide 12.0.

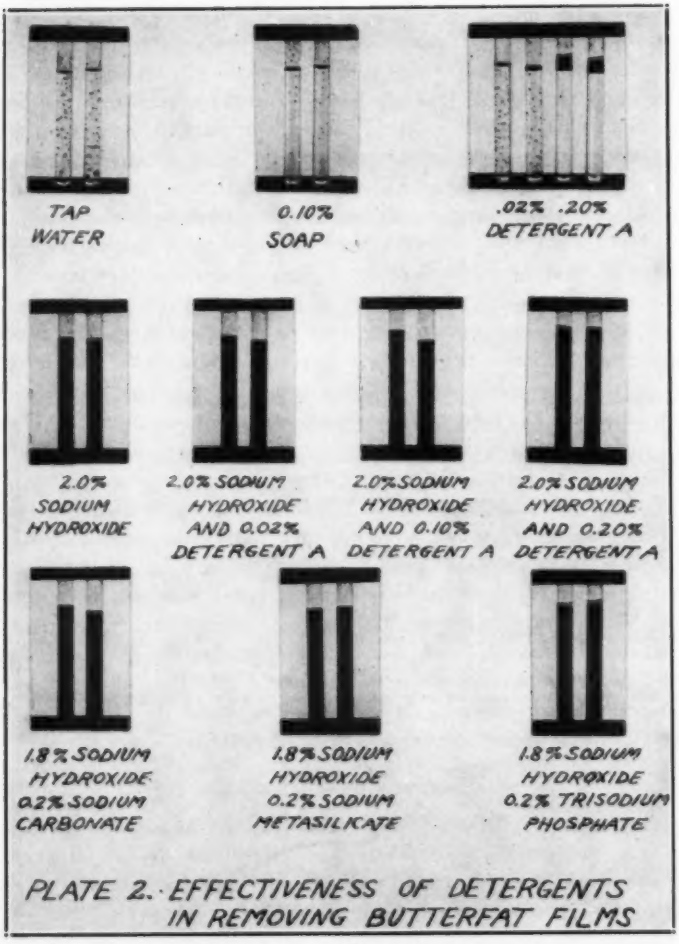

TAP WATER
0.10% SOAP
.02% .20% DETERGENT A
2.0% SODIUM HYDROXIDE
2.0% SODIUM HYDROXIDE AND 0.02% DETERGENT A
2.0% SODIUM HYDROXIDE AND 0.10% DETERGENT A
2.0% SODIUM HYDROXIDE AND 0.20% DETERGENT A
1.8% SODIUM HYDROXIDE 0.2% SODIUM CARBONATE
1.8% SODIUM HYDROXIDE 0.2% SODIUM METASILICATE
1.8% SODIUM HYDROXIDE 0.2% TRISODIUM PHOSPHATE

PLATE 2. EFFECTIVENESS OF DETERGENTS IN REMOVING BUTTERFAT FILMS

3. In the removal of butterfat from glass sodium hydroxide was not effective at any concentration from 0.05 per cent to 2.0 per cent. Sodium carbonate, sodium metasilicate, and trisodium phosphate all were fairly effective above 0.1 per cent.

4. A combination of 1.8 per cent sodium hydroxide and 0.2 per cent sodium carbonate was not as effective in fat removal as similar combinations of sodium hydroxide with sodium metasilicate and trisodium phosphate. The sodium metasilicate mixture produced a greater degree of emulsification than did the trisodium phosphate mixture.

5. Combinations of 2.0 per cent sodium hydroxide and 0.1 per cent and 0.2 per cent detergent A (surface-active organic detergent) were effective in fat removal and similar in action to the trisodium phosphate combination. Detergent A alone was effective at 0.2 per cent but did not produce emulsification.

## REFERENCES

(1) JOHNSON, J. J., AND ROLAND, C. T. Study of dairy cleaning problems. I. Films and deposits on hot-milk equipment. J. DAIRY SC. 23: 457–462. 1940.

(2) BAKER, C. L. Detergent value of sodium metasilicate. Ind. Eng. Chem. 23: 1025–1032. 1931.

# American Dairy Science Association Announcements

## THIRTY-FIFTH ANNUAL MEETING

Plans are approaching completion for the housing and entertainment of the members and guests attending the 35th Annual Meeting of the American Dairy Science Association to be held June 24–28, 1940, at Purdue University, West Lafayette, Indiana.

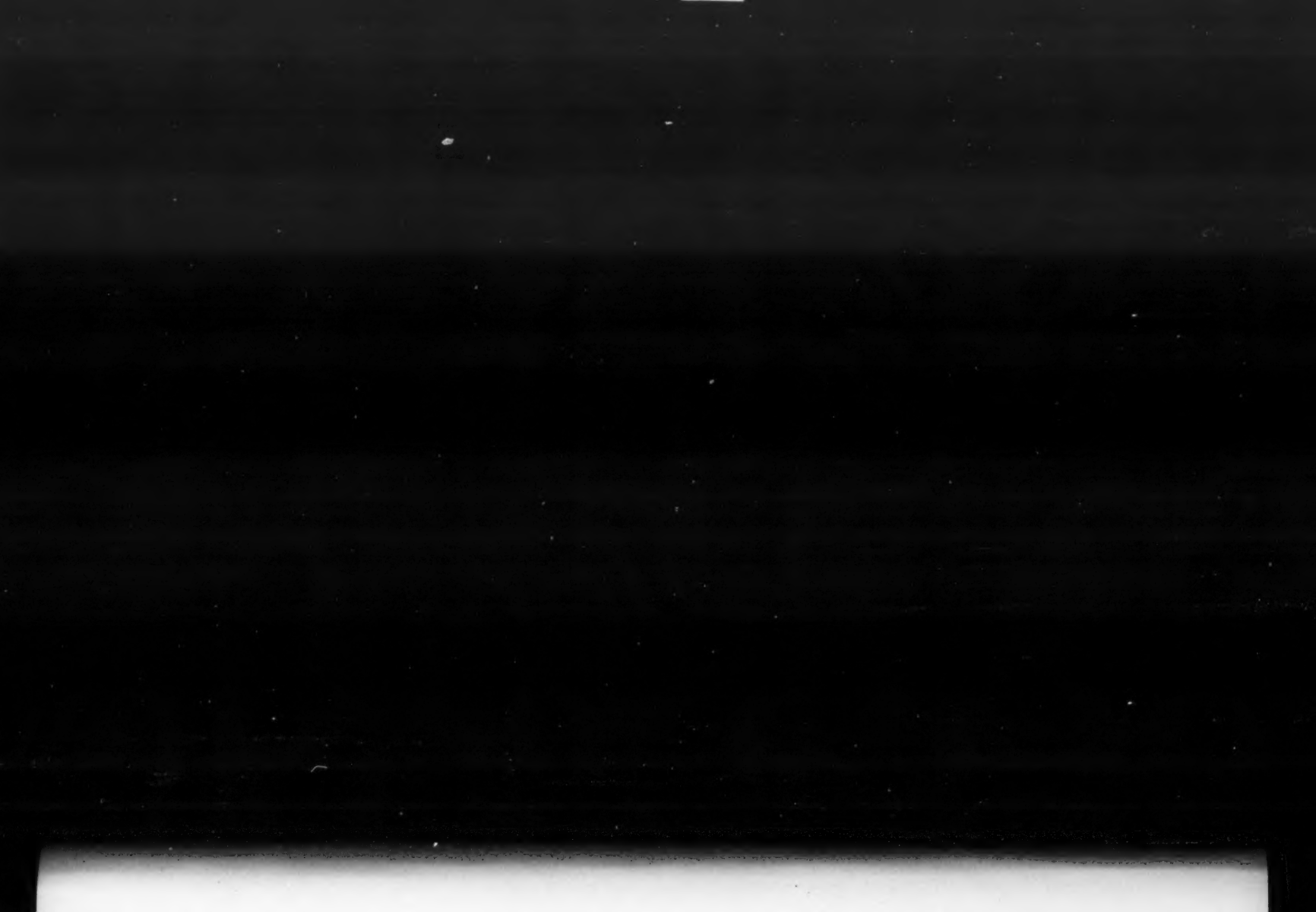
A letter accompanied by a list of housing accommodations and a reservation form has been sent to all members. Accommodations of every kind will be available, including the new Cary Residence Halls, Purdue Memorial Union Club, Hotels in Lafayette, private homes and tourist cabins. To facilitate arrangements, those planning to attend are urgently requested to fill in the reservation form and return it to the Chairman of Registration and Housing before June 1st.

SEE THE JUNE ISSUE OF THE JOURNAL
FOR THE COMPLETE PROGRAM AND ABSTRACTS

Plan now to attend this fine meeting. Bring the family. A program of entertainment is being arranged for the ladies and children. Tours, coffee hours, luncheon bridge and recreational activities are on the program. In addition there are fine recreational facilities available for everyone, golf, tennis, swimming and bowling.

See Purdue University—located on the banks of the Wabash—120 miles from Chicago, 60 miles from Indianapolis, fine roads in all directions.

*Send in your reservations now!*



# ABSTRACTS OF LITERATURE

## BUTTER

**182. Fat Losses in Buttermaking.** DAVID HENRY AND WALTER SLATTER, Ohio State University, Columbus, Ohio. Nat. Butter and Cheese J., *31:* 3, 12. 1940.

Studies determined that fat losses in a roll-less and in a single roll churn of the same make were identical; that pumping cream at 150° F. as compared to 90° F. increased loss of fat; and that percentage of total fat lost decreased with increasing fat content, but increased with increasing acidities of cream measured before neutralization. Losses in typical commercial plants range from 1.21 to 1.48 per cent of the total fat. W.V.P.

**183. Buttermilk Testing.** E. W. BIRD, Iowa State College, Ames, Iowa. Nat. Butter and Cheese J., *31:* 2, 58. 1940.

About 75 per cent of the fatty materials in buttermilk are true fats. Sterols and phospholipins constitute the remainder. Results are presented of twelve fat tests on a single buttermilk sample by the American Association, Babcock, Minnesota and Mojonnier tests. The first and third are recommended because they show greater spread of values as variations in fat occur. Proportion of butterfat losses are approximately calculated by:—

$$\frac{(100 - 1.2 \times \text{cream test}) \times \text{buttermilk test.}}{\text{Cream test}}$$

A graph is presented which shows the relation between results obtained with the different fat tests; and the relation between the per cent of total fat lost, fat in the buttermilk and fat in the cream. The graph shows that proper methods of churning recover about 99 per cent of the milk fat as butter. W.V.P.

**184. Improving the Body of Summer Butter.** S. T. COULTER. Am. Prod. Rev., *88:* 16, 462–463. Aug. 9, 1939.

The outstanding defect of summer made butter is "standing up" properties. Since this is due to a relatively high proportion of soft fats, it may be controlled by regulation of crystallization of butterfat during the processing of the cream. Control consists in cooling and holding cream at the churning temperature, this to be such a temperature that the butterfat will churn in 40 to 60 minutes; and, avoiding overloading of the churn. By such methods firmness of butter may be varied 25 per cent. P.S.L.

**185. New Test for Estimated Size of Water Droplets in Butter.** S. T. COULTER. Am. Prod. Rev., 88: 7, 178. June 14, 1939.

The writer reports the test devised by Knudsen and Sorensen in "Fette and Seifen." To make the test, a piece of Schleiter and Schüll No. 702 Extrahart filter paper is impregnated in a mixture of one ml. normal HCl and 100 ml. of 95 per cent alcohol containing 0.25 grams of bromophenol blue, and then dried. The paper is next held for 30 seconds against the surface of freshly cut butter. The size of the water droplets is indicated by the size of the blue spots which will form on the paper. P.S.L.

**186. Effect of Salt on the Microflora and Acidity of Cream.** D. I. THOMPSON AND H. MACY, Univ. of Minnesota, St. Paul. Nat. Butter and Cheese J., *31:* 2, 12. 1940.

To fresh cream was added salt (NaCl) in 0, 5, 7.5 and 10 per cent concentrations. Portions were then held for 10 days at temperatures of 45° F., 60° F., and 65 to 70° F. In general the results indicate that with increasing salt concentrations in cream, the growth of bacteria and especially yeasts was checked at reasonably low temperatures. Judged by aroma, the quality of cream with 7.5 and 10 per cent salt and stored at temperatures as high as 70° could not be criticized except for slight staleness after 10 days holding. W.V.P.

**187. Variability in Physical Properties of Wisconsin Butter.** K. G. WECKEL, Univ. of Wis., Madison, Wis. Nat. Butter and Cheese J., *30:* 12, 63. 1939.

Magnitude of variations of butter in resistance to crushing, slicing properties, "stand up" properties and "melting point" under kitchen use conditions were determined for 10 Wisconsin factories over a period of a year. Seasonal variations were of a magnitude easily observed by the consumer. Manufacturing methods may account for some variations. Tests for slicing and resistance to softening when served may be employed in creameries. W.V.P.

**188. Wisconsin's Quality Improvement Campaign.** L. G. KUENNING, State Capitol, Madison, Wis. Nat. Butter and Cheese J., *30:* 12, 15. 1939.

The campaign is designed to "help the farmers help themselves." Farmer leaders are trained to present the program in their communities. Proper procedures are explained by experts from the Department of Agriculture and Markets and the College of Agriculture. Plant operators use methylene blue and sediment tests and report farmers delivering milk of inferior quality. State officials enforce sanitary regulations when necessary. W.V.P.

## CHEESE

**189. Blending and Processing.** C. R. Barker. Nat. Butter and Cheese J., *30:* 12, 14. 1939.

A successful blend should have cheese from several factories that has been slowly tempered to 70° F. The blend should be determined according to age and characteristics of the lots of cheese available. Each individual cheese should be plugged if uniformity of lots is uncertain. A good blend consists of 15 per cent current cheese; 15 per cent acid cheese at least 2 months old; 45 to 50 per cent short-held cheese (1 to 3 months of age) on the acid side but not acid; 20 to 25 per cent short-held cheese, sweet and open. Storage cheese (6 months old) can replace short-held in whole or part. W.V.P.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

**190. The Use of Different Sugars in Sweetened Condensed Skim.** George J. Edman, Univ. of Illinois, Urbana. Nat. Butter and Cheese J., *31:* 2, 15. 1940.

Effects of several sugars in the making of sweetened condensed milk are summarized. The sugars considered are sucrose, corn sugar (dextrose), invert syrup and "sweetose," a highly refined corn sugar. The solubilities of the sugars in making and storing condensed milk are compared and combinations of some sugars are recommended to eliminate crystallization. Preservative action of the sugars seems related to but not entirely dependent on osmotic pressure. Color changes in condensed milk are marked when corn sugar and invert syrup are used. Combinations of these sugars with sucrose or use of low storage temperatures or both tend to minimize this defect. Thickening of condensed is particularly noticeable when high temperatures of preheating or high storage temperatures of both are used. Corn sugar and invert syrup especially induce this change. Recommended schedules for the use of corn sugar and "sweetose" are given. W.V.P.

## FEEDS AND FEEDING

**191. Kälberaufzuchtversuch mit erhitzter Vollmilch und natürlichen Vitaminzusätzen.** W. Kirsch. Züchtungskunde, *15:* 1, 18–21. 1940.

At the Albertus University in Königsberg three groups of four calves each were fed from two to seventeen weeks of age on whole milk, untreated for the first group, heated for the second group, and heated but with additions of dry yeast, carrot juice and cabbage juice for the third group. The third group made the fastest and the second group the slowest gains. J.L.L.

**192. Untersuchungen über die Körperentwicklung, Futteraufnahme und Futterverwertung beim roten Höhenvieh.** H. VOGEL UND E. NIX. Züchtungskunde, *15:* 1, 1–17. 1940.

At the University of Giessen seven heifer calves of the red highland breed were weighed each week for their first 26 weeks. Eight different measurements were taken every four weeks. Feed consumption per day and per unit of gain in live weight is given and discussed along with the growth curves. J.L.L.

## FOOD VALUE OF DAIRY PRODUCTS

**193. Milk as a Food throughout Life.** MARGARET HOUSE IRWIN, University of Wisconsin, Madison. Wis. Agr. Exp. Sta. Bull. 447. Nov. 1939.

Science has found that milk more than any other single food meets the nutritional needs of the body. The following table shows nutritive elements in milk and human requirements:

| Nutritional factor | Average daily requirement for a 154 lb. adult | Amount in 1 quart of milk | Approximate portion of the daily requirement supplied by 1 quart of whole milk |
|---|---|---|---|
| Protein | 70 gm. | 31 gm. | ½ |
| Calories | 3000 cal. | 665 cal. | ¼ |
| Calcium | 0.68 gm. | 1.15 gm. | 2 |
| Phosphorus | 1.32 gm. | 0.9 gm. | ¾ |
| Iron | 15 mg. | 2.0–5.0 mg. | ¼ |
| Vitamin A | 3000 to 6000 I.U. | 900 to 1800 I.U. | ¼ |
| Thiamin | 250 to 300 I.U. | 60 I.U. (raw milk) | ⅛ |
| Ascorbic acid | 500 I.U. | 520 I.U. (raw milk) | 1 |
| Vitamin D | 400 I.U. (for children) | 40 I.U. (Summer milk) | ⅒ |
| Nicotinic acid | 25 mg. | Roughly 4 to 12 mg. | ⅛ |
| Riboflavin | 1 to 2 mg. | 2 to 2.5 mg. | 1 |

Milk also contains an exceptional carbohydrate in lactose which has nutritive properties not possessed by other sugars. Lactose digests slowly, favors growth of *L. acidophilus* with consequent acid production in the intestines and favors assimilation of calcium. Milk fat is digested easily and rapidly, is needed for proper utilization of lactose and is of superior nutritive value in addition to its content of vitamins A, D and E. Effects of various processes such as pasteurization on nutritive value are mentioned briefly. The bulletin is written in a popular style and contains 40 pages, 12 illustrations, 1 table and 65 references. W.V.P.

## ICE CREAM

**194. Controlling Distribution Costs.** O'NEAL M. JOHNSON, Int. Assoc. of Ice Cream Mfrs., Washington, D. C. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *3:* 43. Oct. 1939.

In 1938 distribution costs made up 32.8 per cent of the total costs of

ice cream, according to the expense comparisons tabulated by the Statistical and Accounting Bureau of the Association. This segment of cost is almost one-third of the total and herein lies the greatest opportunity for making savings. Products costs are not susceptible to much change and neither are manufacturing costs, which are 21.7 per cent of the total costs. Distribution and sales should be watched closely and kept at a minimum, for unless sales can be made at a profit the plant cannot operate indefinitely. M.J.M.

**195. The Status of Ice Cream Stabilizers.** W. C. COLE, University of California, Davis, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *2:* 35. Oct. 1939.

This is an excellent review of the subject of ice cream stabilizers, their nature and function when used in frozen dairy products. The author summarizes the paper as follows:

"It is known that by properly proportioning the components of ice cream mixes and increasing their total solids one can produce ice cream that is palatable and smooth textured, provided the processing, freezing, and storing are properly controlled. Some manufacturers rely on this method, whereas the majority resort to stabilizers as an aid in controlling quality and as a means of facilitating the production of smooth-textured ice cream. Products such as gelatin and sodium alginate have characteristics that make them desirable for this purpose. Other stabilizers such as agar, pectin, and certain gums seem better suited for ices and sherbets than for ice cream.

"Since the properties of these stabilizers vary, it is generally possible, by selecting and using one or more of them in the correct proportion, to give the finished product certain desirable characteristics that might otherwise be lacking. This fact seems sufficient justification for their use." M.J.M.

**196. Factors Causing Shrinkage in Package Ice Cream.** ROLAND KOHLER, Arden Farms, Inc., Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *2:* 28. Oct. 1939.

There are two types of shrinkage of ice cream in packages. One is severe and is due to a maladjusted mineral salt balance in connection with the peculiar behavior of proteins. A predominance of calcium, or a lack of proper balance between calcium and sodium in relation to phosphates and citrates, will affect the proteins, rendering them unstable. A predominance of calcium may be incorporated from low Bloom gelatin or from some other product. Subsequent high temperature pasteurization and homogenization and freezing will cause curdling of the proteins and shrinkage of the ice cream. The remedy is to adjust the mineral salt balance by adding a small amount of sodium bicarbonate to the cold, fat-free ice cream

mix in the pasteurizing vat. Give the added product a chance to react, then subsequently add an equal or slightly smaller amount of disodium phosphate at a temperature range of 100° F. to 120° F.

The second or less serious type of shrinkage is caused primarily through neglect and inefficiency in operation of machines and too severe application of refrigeration. Excessive homogenization pressures and poor freezing are the usual causes. The result is a gradual sinking or lowering of the ice cream level from the sides and top towards the center of the can. Faulty transportation methods often are to blame for shrinkage of the ice cream. Paper packages and cans, however, seem to be as satisfactory as metal in this respect. High yield is not one of the causes of shrinkage of ice cream. If the product is made from stable ingredients, properly processed and frozen, shrinkage in the package is prevented. M.J.M.

197. **The Preparation of Frozen Fruit Pulps and Their Use in Ice Cream and Related Products.** D. G. Sorber, Assoc. Chemist. U. S. Dept. of Agriculture, Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *2:* 7. Oct. 1939.

Since few ice cream manufacturers are in a position to obtain many varieties of fully ripened highly flavored soft fruit, it is essential that the fruit be properly preserved and stored for later use in ice cream. Such a process described in this article briefly consists of: 1. Selecting full flavored fruit of predetermined suitable varieties; 2. Precooling as an aid to controlling oxidation; 3. Washing; 4. Coarsely crushing or pureeing (depending upon the purpose intended) in such a way as to avoid beating air into the product; 5. Adding a predetermined amount of sugar or syrup and thoroughly mixing to further aid in preventing enzymatic alteration of flavor and color; 6. Packing in tightly sealed enamel-lined tin cans, preferably closed under vacuum; 7. Rapid freezing at sub-zero temperatures; 8. Storing at a temperature of 0° F. or colder.

Rapid handling throughout the entire procedure is advisable to insure the preservation of the maximum quality that exists in the fresh fruit.

The more extensive use of frozen fresh fruit by the ice cream industry is urged as an aid to fruit growers, dairymen and ice cream manufacturers. The consumer, as well, will benefit from increased variety, greater appeal and higher quality. M.J.M.

198. **The Northwest Association's Cooperative Laboratory Project.** H. Macy, University of Minn., St. Paul, Minn. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *2:* 20. Oct. 1939.

In 1938 the Northwest Association decided to: (1) adopt purchase standards for ice cream ingredients, (2) meet annually for a two-day conference of instruction, and (3) establish a cooperative laboratory to give an

opportunity for its members to have bacterial and chemical analyses made at a reasonable cost.

The standards set for the quality of ingredients to be used in ice cream are given in this article. The annual conference has proved successful and the cooperative laboratory has proven to be an economical venture. Among the various ingredients examined, gelatin has given counts exceeding the purchase standards in 66 per cent of the samples; chocolate products in 61.3 per cent; egg yolk in 47.5 per cent; nuts in 42 per cent; and strawberries in 36 per cent. All of these have frequently given positive tests for coliform types. Over 30 per cent of the colors and fifty per cent of the flavors were unsatisfactory and several candy products have been too high in bacterial content.

The results obtained to date indicate that the subscribers are making considerable progress in manufacturing frozen products which are satisfactory from the standpoint of chemical composition and sanitary quality. They are becoming more discriminating in their purchase of raw dairy products and miscellaneous ingredients. A much closer check is being made of processing and plant sanitation. So far the laboratory has proved to be a most useful agency for furnishing technical information to all manufacturers cooperating in the project. Plants having difficulty at the start are solving their problems very adequately. The future holds much promise of marked improvement when definite facts are available at all times for the enterprising manufacturer of ice cream. M.J.M.

199. **Merchandising Ice Cream.** JOHN C. MILTON, Hudson Mfg. Co., Chicago, Ill. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *4:* 10. Oct. 1939.

This article is a discussion of the subject, "Merchandising at a Profit." The principal points in successful merchandising are discussed in an effective manner by the author. M.J.M.

## MILK

200. **Significance of Coli in Milk.** H. J. BRUECKNER. Am. Prod. Rev., *88:* 27, 838–839. Oct. 25, 1939.

The presence of E. coli in average raw milk is not significant, but if present in pasteurized milk indicates improper pasteurization or contamination after pasteurization. Recent studies at Cornell University show that less than one per cent of colon organisms survive pasteurization. Contamination after pasteurization may result from unsterilized apparatus, especially no-foam can fillers, rubber on bottle filler valves, and air tubes on bottle fillers. P.S.L.

201. **Increasing Viscosity of Cream.** H. J. BRUECKNER. Am. Prod. Rev., *89:* 9, 237. Dec. 27, 1939.

Two methods are outlined for increasing the thickness of bottle cream. By the first the milk, after pasteurization, is cooled to 70 to 75° F. before separation. With newer type separators temperatures as low as 65° F. may be used. Since temperatures affect richness of cream the cream screw must be adjusted accordingly. The second, more effective method, consists in cooling the milk, after pasteurization, to 50° F. or lower, holding at that temperature from two hours to overnight, warming to 80–84° F., and separating. Fat losses in skimmilk will vary from 0.01 to 0.05 of one per cent and bacterial count may increase very slightly. P.S.L.

202. **A Study of Oxidized Flavor.** ALBERT S. TOMLINSON. Am. Milk Rev., *2:* 2, 34–35. 1940.

This article is a condensed popular version of the author's thesis on the same subject. An unsuccessful effort was made to find milk which would develop oxidized flavor without addition of copper. Some samples were found developing the flavor with additions of 0.1 p.p.m. of copper. The author was unsuccessful in proving involvement of an enzyme in the reaction, although inhibition of the flavor development by high heat treatment would seem to support the theory of its role. He suggests, from results of his data, that failure of the defect to appear after high heat treatment may be due to chemical changes in the albumin of milk. P.S.L.

203. **Effect of Heat on Milk with Especial Reference to the Cooked Flavor.** I. A. GOULD, JR., AND H. H. SOMMER. Mich. Tech. Bull. 164. May 1939.

Studies were conducted to determine the influence of various factors upon the cooked flavor of milk, to ascertain the relationship of the cooked flavor to oxidation-reduction potentials and to oxidized flavor development, and to determine, if possible, the cause of the cooked flavor. Under the conditions of this experiment, the cooked flavor of milk occurred normally when the milk was heated momentarily to 76–78° C. This temperature was decreased with appreciable increases in the fat content, the pH, or the holding period, and upon addition of small quantities of sodium sulfite. The cooked flavor temperature was slightly increased by homogenization of the milk prior to heating, by lowering the pH, and by addition of ferrous iron at the rate of 1.4 to 2.8 p.p.m. The temperature at which cooked flavor appeared was markedly raised by the addition of one p.p.m. of copper to the milk before heating.

Decreases in the oxidation-reduction potentials were found to occur when milk was subjected to relatively high temperatures, this decrease being

closely correlated with the appearance of the cooked flavor. In addition, the heat retardation and prevention of copper-induced oxidized flavor was found to be related to the cooked flavor, especially when the copper was added before the milk was heated. The critical temperature range in this connection was approximately 84–86° C. However, when the copper was added following the heat treatment, the cooked flavor quickly disappeared and the milk became oxidized even though temperatures of 90° C. were used for processing the milk.

The liberation of sulphides from milk, probably as hydrogen sulphide, was found to be closely correlated with the appearance of the cooked flavor and the lowering of the oxidation-reduction potential. These sulphides also served to explain the effect of heat on oxidized flavor development.

Efforts to utilize the nitroprusside test as a means of detecting sulphydryl groups in heated milk were unsuccessful. P.S.L.

**204. Curd Tension of Chocolate Milk Drinks.** G. HADARY AND H. H. SOMMER, Univ. of Wis., Madison, Wis. Milk Dealer, *29:* 3, 42. Dec. 1939.

It is shown that a low curd tension is characteristic of chocolate milk drinks. In part the reduction in curd tension is due to the higher pasteurizing temperatures used in making the products. However, the main effect is due to the cocoa itself. The effect of sugar and suspending agents such as starch, locust bean gum and "cocoloid" is negligible. C.J.B.

**205. Processing and Handling of Coffee and Whipping Cream.** M. J. MACK, Mass. State College, Amherst, Mass. Milk Dealer, *29:* 3, 38, 46–48. Dec. 1939.

The following defects of cream and their prevention are briefly discussed: 1. Poor flavor. 2. Poor keeping quality. 3. The formation of cream plug. 4. Poor body. 5. Serum separation. 6. Oiling off in coffee. 7. Feathering in coffee. Factors affecting the whipping ability of cream are also briefly discussed. C.J.B.

**206. Preventing Development of Oxidized Flavor in Milk.** E. O. ANDERSON, Dept. of Dairy Industry, Univ. of Conn., Storrs, Conn. Milk Dealer, *29:* 3, 32, 82. Dec. 1939.

Some of the practices which are used to prevent or minimize the development of oxidized flavor in milk are listed. The case histories of five plants which have prevented the development of oxidized flavor by the use of pancreatic enzyme are given.

The author summarizes the use of the enzyme in commercial plant practice as follows: "It can be said that it offers a reliable relief from

trouble with oxidized flavor, whether the difficulty is due to metal contamination or to naturally high susceptibility of the milk. Pancreatic enzyme appears to be specific for the prevention of the development of oxidized flavor." C.J.B.

**207. The Place of Plate Heat Exchange Equipment in the Dairy.** GLENN E. WEIST, Dairy Engineer, Chicago Ill. Milk Dealer, *29:* 3, 30, 31, 78–80. Dec. 1939.

A brief discussion of the development of plate heat exchange equipment followed by examples showing how economies can be effected by regeneration. The economies are based on reduction in heating load, reduction in cooling load, and freedom from obsolescence.

In conclusion the author states that: "It seems reasonable to conclude that plate heat exchange equipment should receive consideration in all heating and cooling operations; that it offers a possibility of an improved product through processing in a closed system that is more accessible for cleaning; and finally, that it offers many striking economies in operation, flexibility plus freedom from obsolescence." C.J.B.

**208. Photochemical Study of the Irradiation Process of Producing Vitamin D Milk.** M. J. DORCAS. Milk Dealer, *29:* 4, 64–68. Jan. 1940.

A discussion of what takes place when milk is irradiated. Different types of irradiators and their efficiency are also discussed. C.J.B.

**209. The Control of Sediment in Homogenized Milk.** A. J. HAHN AND P. H. TRACY, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. Milk Dealer, *29:* 4, 58–60. Jan. 1940.

A discussion of the causes and control of sediment in homogenized milk. The authors state that in order to eliminate sediment in homogenized milk, the following points should be considered:

If bottled homogenized milk is left standing at temperatures between 40° and 45° F. without excessive agitation, and if the milk is normal in every respect, then it will be possible to eliminate sedimentation when the milk contains approximately 100,000 ± 25,000 cells per ml.

If the temperature of the milk has been allowed to increase at any time after it has been bottled, and if there has been agitation of these bottles of milk at these higher temperatures, then a cell count below approximately 50,000 cells per ml. is necessary to eliminate sediment.

Clarification or separation is necessary when milk of a cell content above 125,000 per ml. is being used for homogenizing purposes. Repeated clarification will not only reduce the cell content of the milk after each clarifica-

tion, but will permit the use of milk with a higher cell content than that required of single clarification. Clarification after homogenization is slightly more efficient than clarification before homogenization.

Destabilization of the milk protein will increase the amount of sediment while stabilization will decrease it. C.J.B.

**210. Suggestions on Selling Milk through Vending Machines.** Anonymous. Milk Dealer, *29:* 4, 44. Jan. 1940.

Two milk dealers, one of St. Louis, Missouri, and the other of Louisville, Kentucky, discuss the introduction of milk-vending machines. In large cities where drivers are unionized, they advise sticking to the half-pint bottle rather than trying the third-quart bottle. Suggestions for locating and operating vending machines are also given. C.J.B.

## MISCELLANEOUS

**211. What's New in Farm Science.** Anonymous. Part One, Annual Report of the Director Agr. Exp. Sta., Univ. of Wis., Madison, Wis. Bull. 446. Nov. 1939.

This report presents in brief the results of work completed and in progress at this station. In the table of contents, page 95, are listed the following titles of interest to the readers of the JOURNAL OF DAIRY SCIENCE:

Animal Diseases and Breeding: Make new findings on use of hormones for breeding troubles; Does heredity control response to hormones; Determine best time for insemination of dairy cows; Is linebreeding practical with dairy cattle; High producing young cows increase production most.

Animal Nutrition: Whole milk is better than filled milk with added vitamins; Animals thrive when kept on mineralized milk only; How much would feeding grass silage improve market milk; Here's what's new in grass and legume silage; Learn more about the effects of spoiled sweet clover; Urea gives good results as a protein substitute in calf rations; Learn why animal proteins improve soybean oilmeal rations; Egg whites must contain plenty of riboflavin if the eggs are to hatch; New method cuts the cost of assaying feeds for riboflavin; Running fits in dogs can be prevented by proper diet; How do common foods compare in nicotinic acid content; Seek to isolate the grass juice vitamin; Cast new light on the value of copper in treating anemia.

Bacteria, Molds, and Yeasts: Develop quick method of producing lactic acid; Swiss cheese starter may be kept 24 hours.

Dairy Products: How closely can the fat content of Swiss cheese be controlled; What starter combinations are best for Brick cheese; Cream testing 29 per cent has advantages for buttermaking; Are propionates or propionic acid useful in buttermaking; Some treated wrappers improve

keeping quality of storage butter; Sodium alginate proves useful in dairy manufacturing; Improve test for pasteurization efficiency; Lecithin content of milk is lowest in strippings.

Farm Income and Welfare: Test of milk going to cheese factories varies considerably; Dairy cooperatives need efficient organization; Should milk be made a public utility? W.V.P.

212. **What Shall I Use for Fuel in My Dairy Plant?** S. KONZO, Engineering Experiment Station, Univ. of Ill., Urbana, Ill. Milk Dealer, *29:* 4, 50. Jan. 1940.

An arbitrary classification of the merits of various fuels and the calculated comparative costs of fuels are given.

The author then presents the following 14 methods by which maximum combustion efficiency may be obtained:

1. Provide air-tight flue passages. Seal leaks in boiler setting, smoke pipe, and chimney.

2. Provide sufficient, but not excessive draft to carry away flue gas products. Use automatic draft-regulating dampers to maintain minimum required draft.

3. Keep heating surfaces clean. Periodically remove soot accumulation from flue passages and smoke pipe. Clean out scale formation on water side of heating surfaces.

4. Inspect baffles in combustion chamber to see whether they have fallen or are leaky.

5. Maintain adequate and proper distribution of air for the combustion process. $CO_2$ percentages of the following amounts may be considered as acceptable; coal, 12 per cent; oil, 10 per cent; gas, 10 per cent.

6. Maintain smoke-free combustion. In the case of oil a slightly hazy atmosphere in the combustion chamber will usually accompany proper combustion. In the case of coal a slight trace of smokiness and a slightly yellow flame is a good visual index to use.

7. If possible, use a boiler specially designed to burn the fuel to be used. A coal-burning boiler when converted to oil burning should be properly baffled and should be equipped with special combustion chambers.

8. In all cases provide a rate of burning that is just sufficient to handle the maximum demands. Excessive burning rates may result in rapid pick-up, but also result in excessive flue-gas temperatures and large flue losses.

9. Provide adequate and trustworthy thermostatic and pressure controls for the safe regulation of the combustion process.

10. In the case of hand-fired, coal-burning plants, fire the coal into the combustion chamber so that the fresh charge does not completely cover the entire fuel bed. Live coals should be exposed so that volatile gases will ignite. Remove ashes from the ash-pit to prevent burning out of the grate bars. Egg-sized and nut-sized coals should be used.

11. In the case of stoker-fired plants, remove clinkers periodically from the fuel bed. Stokers will not satisfactorily handle any old coal. Best results are obtained with coals specially prepared for stoker use. This preparation includes sizing (1-inch, 1½-inch or 2-inch coals are used, depending on the size of the stoker), washing, and oil treating of the coal.

12. In the case of oil-fired plants use clean oil of the quality recommended for the specific burner. Periodic and frequent inspections of the oil strainer, burner nozzle, and ignition device should be made.

13. In the case of gas-fired plants, provide proper and constant gas pressure, reliable pilot lights, and fool-proof safety controls. Periodic inspection of the chimney should be made to determine whether any flue-gas condensation may be damaging the chimney or smoke pipe.

14. Provide boiler plants with draft gauges, flue-gas-temperature recorders, and some means for checking the $CO_2$ in the flue-gas, either periodically or intermittently. C.J.B.

**213. Internal Audit Control.** CHARLES W. TUCKER, H. P. Hood & Sons, Boston, Mass. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *3:* 54. Oct. 1939.

Mr. Tucker summarizes his discussion by stating that a satisfactory state of internal audit control may be said to have been achieved when the factors of organization, personnel, policies, and procedures, records and mechanical and other equipment aids have been so developed and combined as to produce a harmonious whole. This coordinated effort should make possible an "Earnings Account" with a credit balance commensurate with what management and stockholders might reasonably expect as a return on their investment of effort and capital. M.J.M.

**214. Marquis of Queensbury Rules for Modern Business.** I. E. LAMBERT, Attorney at Law, Santa Fe, New Mexico. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *3:* 28. Oct. 1939.

This is a discussion of trade practices, which in the opinion of the author, hinder business. In addition he discusses possible future legislation which should be followed with keen interest in order to see that it is practicable and workable. M.J.M.

**215. Executive Use of Accounting.** PAUL H. ANDRES, The Central Dairy Products Co., Oklahoma City, Okla. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *3:* 8. Oct. 1939.

The writer states that the responsibility of an accounting department is to give to management what it needs and wants. Management should accept accounting reports as accurate statements of facts, containing the

clews to bad operating and other conditions of business. If the accounting department is competent, and if management realizes the functions of accounting and is willing to use all the information it gets, then the executive and accounting departments will continue the progress already made, and better operating results will be deprived from these efforts. M.J.M.

216. **Why Sales Are Lost.** FRANK WARREN, Arden Farms, Inc., Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *4:* 22. Oct. 1939.

There is a reason for losing sales, and when the cause is known a remedy may be found. Sales are lost because of indifference, delay in serving the customer, mistakes, worry, ignorance, a poor quality of merchandise, lack of cleanliness, lack of personality, ungratefulness, and price. The author considers each of these causes separately and offers solutions for preventing loss of sales by any of these factors. M.J.M.